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2838

Phenoltetrachlorphthalein test of a liver function in Eck fistula dogs kept upon meat diet.

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The chemical studies of phenoltetrachlorphthalein by Orndorff and Black[1] in 1909 were followed by the pharmacological investigations of Abel and Rowntree[2] in the same year. Subsequently, in 1913, Whipple, Peightal and Clark[3] studied the excretion of the dye under experimental conditions. Included among the various types of liver injuries which they produced in dogs were three Eck fistulas. In one of the dogs, the phthalein output was not impaired at the end of twenty-three days after the establishment of the fistula; in another, there was no impairment at the end of one year; in the third, the excretion was low for some weeks after the operation but reached normal values at the end of four months. The determinations were made by the cumbersome and inaccurate method of recovering the dye from the feces and

[1] Orndorff, W. R., and Black, J. A., *Am. Chem. J.*, 1909, xli, 349.

[2] Abel, J. J., and Rowntree, L. G., *J. Pharmacol. and Exper. Therap.*, 1909, i, 231.

[3] Whipple, G. H., Peightal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 343.

urine during a period of twenty-four hours after the injection. Since this work, nothing has appeared in the literature concerning the ability of the liver of Eck fistula dogs to eliminate phenoltetrachlorphthalein.

In 1922, Rosenthal[4] introduced a simpler and more accurate method of estimating liver function by determining the rate of removal of phenoltetrachlorphthalein from the blood stream. This method was employed in the following study upon Eck fistula dogs.

Seven dogs were used in the experiment. First, an anastomosis was made between the vena cava and the portal vein. Then, the portal vein was ligated above the anastomosis and above the entrance of the vena pancreatico-duodenalis.

The technique* which was employed was a modification of Eck's[5] original operation described by Guleke,[6] and Bernheim and Voegtlin.[7]

The test was performed according to Rosenthal's description, at periods varying in the different dogs from forty-six to one hundred thirty-six days after the establishment of the fistulas (see chart). During the twenty-four hours immediately preceding the test, the dogs received no food. Water was allowed *ad libitum.* Six cubic centimeters of blood were withdrawn from one jugular vein for the standard of comparison and then five milligrams of the di-sodium salt of phenoltetrachlorphthalein per kilo of body weight were injected into the same vein. Fifteen minutes and again one hour after the injection, six cubic centimeters of blood were removed from the other jugular vein and the amount of the dye retained in the serum was determined by comparison with colormetric standards.

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[4] Rosenthal, S. M., *J. Pharmacol. and Exper. Therap.*, 1922, xix, 385.

* The surgical procedures were performed in the laboratory of the Department of Experimental Surgery of the College of Physicians and Surgeons.

[5] Eck, von N., *Militär-med. Journ.*, 1877, lv, 130.

[6] Guleke, N., *Ztschr. f. Exper. Path. u. Therap.*, (Berlin) 1906, iii, 706.

[7] Bernheim, B. M., and Voegtlin, C., *Bull. Johns Hopkins Hosp.*, 1912, xxiii, 46.

Dog Number	*Per cent of dye in blood serum at the end of 15 minutes.	*Per cent of dye in blood serum at the end of one hour.	Duration of Eck fistula at time of test. Days	Days on meat diet at time of test.	Total duration of Eck fistula at time of autopsy.
I	4%	3%	136	84	155
II	4%	4%	119	86	136
III	3%	3%	114	73	124
IV	4%	3.5%	102	71	166 (no autopsy)
V	2%	3%	99	78	136
VI	3%	3%	71	70	159
VII	3%	3%	46	36	130

* We are indebted to Dr. L. Bauman for corroborating these determinations in his laboratory at the Presbyterian Hospital.

As indicated in the chart, the quantity of the dye recovered from the blood serum at the end of fifteen minutes varied between two and four per cent. At the end of one hour the amount was from three to four per cent. These results are within the range of normal values. They indicate that the rate of disappearance of the phenoltetrachlorphthalein was rapid and that the ability of the liver to remove the dye from the blood was not impaired.

The dogs were kept upon a diet of lean meat which was started at intervals of one to fifty-two days after the operation, and was continued up to the time of exitus. Contrary to the observations of Pawlow and his co-workers,[8] Fischler,[9] and others, none of the dogs in the above series exhibited nervous symptoms.

Dogs III, IV, V, and VI became greatly emaciated and died one hundred and twenty-four to one houndred and sixty-six days following the establishment of the Eck fistula (see chart); Dog VII, which had lost considerable weight, was killed after one hundred and thirty days because of mange. Dogs I and II were used for other purposes after the dye injection; subsequently they were killed and examined.

Autopsies were performed upon all of the dogs except dog IV. Examination uniformly revealed that communications had been established between the vena cava and the portal vein, and that the latter had been completely occluded above the entrance of the vena pancreatico-duodenalis. There were no collaterals. The

[8] Hahn, M., Massen, O., Nencki, M., and Pawlow, J., *Arch. f. Exper. Path. u. Pharmakol.*, 1893, xxxii, 161.

[9] Fischler, F., *Deutsch. Arch. f. Klin. Med.*, 1911, 104, 300.

livers of the Eck fistula dogs showed definite changes which will be described later.

Conclusions. The ability of the liver to remove the di-sodium salt of phenoltetrachlorphthalein from the blood stream was not impaired by the establishment of Eck fistulas in dogs. Definite lesions in the liver (to be described later) followed the establishment of the fistulas. A prolonged meat diet did not induce toxic symptoms in these dogs.

2839

Antiscarlatinal serum of dual potency.

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The question of the complex nature of hemolytic streptococcus antiserum has been studied since the time of Van deVelde,[1] Marmorek[2] and others.

Protective power was early demonstrated. Antitoxic powers were also demonstrated broadly for the scarlet fever strains by Moser,[3] Savchenko[4] and others, and were recently made clear cut by the work of Dochez[5] and of the Dicks.[6]

Realizing that a serum having the power to protect against the living culture, as well as, to neutralize the toxin, might have advantages over a strictly antitoxic serum, we have been inoculating several of our horses by what must be called a modified Moser method. The modifications have been learned by our work on diphtheria antitoxin and antipneumococcus serum, that is, that a protective serum is obtained most easily by the repeated inoculation of the whole killed culture into the vein, and that the antitoxin serum is obtained by the repeated inoculation of toxin subcutaneously.

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[1] Vandevelde, *Arch. de Med. Exp.*, 1897, ix, 835.
[2] Marmorek, Alexandre, *Am. de L' Inst. Past.*, 1895, ix, 593.
[3] Moser, *Wien. Klin. Woch.*, 1902, xv, 1053.
[4] Savchenko, *Russk. Vrach.*, 1905, xxv, 797.
[5] Dochez, A., *J. Am. Med. Ass'n.*, 1924, lxxxii, 542.
[6] Dick, G. F., and G. H., *J. Am. Med. Ass'n.*, 1924, lxxxii, 1246.

We inoculated the killed whole washed cultures intravenously and the Buchner filtrates of a six day growth in blood bouillon subcutaneously. Cultures of the filtrates showed 100 colonies of the streptococcus to the cc.

One of the horses we inoculated in this way, No. 86, has been under treatment for nine months. The others for much shorter times.

The inoculations into this horse were started on January 15th, 1925, by giving the whole centrifuged 24 hour broth cultures intravenously. Two days later the Buchner filtrate of a six day blood broth culture was started subcutaneously. The doses were continued on alternate days and were gradually increased. In about 3 months the serum began to show blanching in the Schultz Charlton test. In 5 months the serum was strongly blanching, and 1 cc, neutralized 10,000 Dick skin test doses of the streptococcus toxin. At this time it showed no definite protection.

After nine months 1 cc. neutralized 40,000 skin doses of toxin and 0.05 cc. protected mice against 100 M.L.D. of very virulent living streptococci.

The clinical results with this serum have been excellent but it is impossible yet to state that they have been better than those obtained with antitoxic serum alone. Theoretically such a dual serum should not only neutralize the toxic effects of the streptococcus in scarlet fever but should reduce the number of so-called complications and sequellæ of this disease.

2840

The Specific Dynamic Action of Carbohydrates.

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The speed with which glucose or sucrose may be absorbed and metabolized when it is administered orally would seem to be rapid, judged from observations such as the time necessary for the recovery from insulin hypoglycemia after taking glucose by mouth. No experimental data are on record, however, in which

an investigation of this point has been carried out by the method of determining how soon the R. Q. becomes elevated from increased carbohydrate utilization or how soon the specific dynamic action becomes manifest after carbohydrate ingestion in normal animals. In the experiments of Lusk[1] on the specific dynamic actions of the common sugars, carried out on dogs in the respiration calorimeter at Cornell University Medical College, technical difficulties have prevented a determination of the effects of the carbohydrate during the first hour following its administration.

The experiments described below were conducted by making use of the open circuit type of respiration apparatus, which allowed metabolism determinations in the interval immediately following the ingestion of the different carbohydrates. The experiments were carried out on one of us (H. J. D.). The majority of these experiments were done during a period in which the subject was taking a protein-free diet and the others somewhat later during a period of low protein diet. Two basal determinations of 15 minute intervals were carried out each morning before the taking of 75 grams of the carbohydrate under investigation. After the carbohydrate was ingested a series of 11 or 12 determinations of metabolism were performed in the following period of 4¾ or 5¾ hours. Four of these were carried out during the first hour.

When sucrose or fructose was given orally an increased R. Q. was noted in the first 10 minute period thereafter, while during the second 10 minute interval the R. Q. usually reached the maximum observed during the experiment, often being greater than 1.00. A slightly slower response was obtained in the rate of elevation of the R. Q. with galactose and lactose. However, after the ingestion of glucose or maltose only a slight increase in the level of the R. Q. occurred during the first 30 to 45 minutes, and the rise which followed was usually less than in the case of sucrose, fructose or galactose. When raw starch was ingested little effect on the R. Q. became evident until the second hour, the maximum quotient of 0.90 being reached in the third hour. With cooked starch the curve of the R. Q. resembled that of glucose when the starch was given as a pudding; on the other hand, the absorption and utilization of the dried, cooked starch was mark-

[1] Lusk, G., *J. Biol. Chem.*, 1915, xx, 555.

edly delayed, as judged by the R. Q. obtained after its administration. The results obtained with glucose and fructose with regard to the R. Q. agree with the unpublished results of Wierzuchowski and Boothby.[2]

The specific dynamic action reached a maximum between two and two and one-half hours after the ingestion of 75 grams of the sugars studied here. The maximum heat production usually was coincident with the highest R. Q., although this was not invariably the case. At the end of 4½ hours the increased heat production had usually passed, the metabolism had fallen to the basal level, and the R. Q. had returned to the fasting level.

In the case of raw starch and dried cooked starch, although progressively increasing quotients were obtained during the first two hours, indicating an increasing utilization of carbohydrate, only slight changes of basal metabolism occurred. This indicated that the slow absorption did not result in a plethora metabolism, although sufficient glucose was absorbed to increase the R. Q. appreciably.

The amount of the specific dynamic action of lactose, which Lusk has found to be small in dogs in comparison to the other sugars, was found to be approximately equal to that of the other sugars with man.

2841

Ultra violet rays in the purification of cultures of spirochaeta pallida.

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During the past few years, we have been engaged in an effort to improve existing methods, whereby cultures of *Spirochæta pallida* may be freed from contaminating bacteria, thus permitting more readily the isolation of the former organism. In the present investigation, it was our aim to test the selective bacteri-

[2] Personal communication.

cidal action of ultra-violet rays with the hope that this would accomplish our purpose.

To the best of our knowledge, no literature has yet appeared on the action of the ultra-violet rays on spirochætes in general or on the *Spirochæta pallida* in particular. The nearest approach to this problem was made by Rosenberger and Fanz,[1] who studied the effects of the Roentgen ray and radium on *Spirochæta pallida.* They found that fourteen day cultures of the spirochæte, exposed to full erythema doses of the X-ray (60 milliamperes, *i. e.*, five milliamperes for twleve minutes) showed no tendency to deterioration, but retained their activity and typical morphology. The transplants grew vigorously and did not die upon subsequent sub-cultures. Similar results were obtained upon exposing the cultures to radium for one hour. Even a full erythema dose of the Roentgen ray followed by a full erythema dose of radium failed to check the reproductive powers or the morphological characteristics of the organism. Rosenberger concludes, therefore, that the "X-ray and radium, in dosage harmless to man, possess no power to destroy this peculiar and interesting parasite."

Method of Investigation.

A number of preliminary experiments which need not be detailed here were carried out before the following technic was finally adopted. It will be noted that quartz glass (because of its great cost) was not used in this work, thus reducing much of the germicidal action of the ultra violet rays. The culture media, as well as other details of the methods used in these experiments have been described in a previous communication.[2]

Technic.

In a series of small sterile test tubes were placed 0.75 cc. of a stock culture of *Spirochæta pallida* grown on a modified Schereschewsky medium. To each tube was added one drop of a twenty-four hour bouillon culture of the contaminating organism to be tested. *Staphylococcus aureus* and *Bacillus coli* were the two types used, as representative of the Gram positive and

[1] Rosenberger, R. C., and Fanz, J. I., Researches from the John H. McFadden Research Laboratory of the Jefferson Medical College, Philadelphia, Pa., 1919.

[2] Weiss, C., and Wilkes-Weiss, D., *J. Inf. Dis.*, 1924, xxxiv, 212.

Gram negative groups of bacteria. Each tube was then exposed to ultra-violet radiations as projected from the Alpine Sun Lamp* for an allotted time and distance range. The apparatus was allowed to reach its maximum intensity by burning the lamp for fifteen minutes before exposing the tubes. The time intervals used were from fifteen seconds to sixty minutes, and the range varied from close application of the quartz lamp to a distance of 10 centimeters or more. The exposed material was now planted on horse serum medium, incubated at 37°, for fourteen days, and examined by dark field illumination (Leitz-Wetzlar oil immersion lens, 1/12 objective) for spirochætal growth, and by means of agar slants for bacteria.

As the work progressed, it occurred to us that contamination at the human or rabbit source of the *Spirochæta pallida* is not usually as great as was the number of bacteria added to our cultures of spirochætes. This led to the following experiments: One drop of a 1:10, 1:50, or 1:100 dilution of the bacterial growth (in double distilled water) was used instead of the pure culture. This series also includes the use of one drop of a mixture of equal quantities of bouillon growths of *B. coli* and staphylococci, as contaminating organisms.

Summary of Results.

The action of ultra-violet light when projected through thin walled small test tubes containing mixtures of *Spirochæta pallida* and various bacteria may be summarized as follows: With an exposure of 75 seconds at close range, Colon bacilli were killed and treponemata showed good growth. This growth diminished as the time of exposure was increased. Upon re-inoculating on to the fresh media, the spirochætes exhibited good procreative and normal morphological properties, with no evidence of bacterial contamination. A five to ten cm. projection distance was lethal to the spirochætes within 45 to 60 minutes. One hour's radiation at a distance of 10 cm. did not prove bactericidal, but with the lamp placed five cm. away from the tubes and allowed to act but 45 minutes, the Colon bacillus was effectively destroyed.

When a definite interval of exposure to ultra-violet rays was selected, it was observed that the lighter suspensions of bacteria

* We are indebted to Doctor J. F. Schamberg, the director of this Institute for the use of his "Alpine Sun Lamp" apparatus.

TABLE I.

Action of ultra-violet rays on cultures of *Spirochæta Pallida* and Colon or Staphylococci (in dilute suspensions). Lamp at close range.

Organism	Dilution of Bacterial Culture	Time Exposure	Dark Field Examinations	Bacterial Growth
Colon Bacillus	1:10	60 seconds	++++	+
		65 "	+++	0
		70 "	+++	0
		75 "	++	+
		80 "	+++	0
		90 "	+++	+
	1:50	60 "	++	0
		65 "	+	0
		70 "	±	0
		75 "	+++	+
		80 "	+++	+
		90 "	++	0
	1:100	60 "	0	0
		65 "	++	0
		70 "	++	+
		75 "	+	0
Staphylococcus Aureus	1:10	60 "	++	0
		70 "	++	0
		80 "	+	0
		90 "	±	0
	1:50	60 "	+++	0
		70 "	++	0
		80 "	+	0
		90 "	0	0
	1:100	60 "	±	+
		70 "	+	+
		80 "	+	0
		90 "	0	0
Colon Bacilli and Staphylococci	1:10	60 "	+	Colon +
		70 "	+	and Staph. +
		80 "	+	0
		90 "	0	0
		120 "	Lost	0
	1:50	60 "	±	0
		70 "	0	0
		80 "	++	0
		90 "	0	0
		120 "	0	0
	1:100	60 "	+	0
		70 "	+	0
		80 "	++	0
		90 "	0	0
		120 "	0	0

++++ excellent growth ++ fair ± occasional spirochæte
+++ good growth + poor 0 no growth

were more readily killed than heavier ones. In other words, this germicidal phenomena is quantitative. It was also noted that the further the source of the rays was from the organisms, all other things being equal, the less the bactericidal action. The longer the time of action, the greater the danger to both the bacteria and spirochætes. A period of exposure of 90 to 120 seconds at close range consistently proved detrimental or lethal to the pallida. Up to an interval of one minute, they were little affected.

2842

Experimental studies on the formation of Hassall's corpuscles.

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This work was undertaken with the hope that a study, by the method of autoplastic transplantation, of the regenerative changes in the thymus gland of an animal like the guinea pig, whose thymus normally contains large numbers of Hassall's corpuscles, would yield some conclusive information concerning the origin of these structures.

Since 1846 when Hassall[1] described these corpuscles, they have held the attention of histologists, embryologists, and pathologists who have worked with this gland. It is interesting, that even today, in spite of the great number of studies dealing with the thymus gland, there is no unanimity of opinion in regard to the origin and possible function of these corpuscles.

A review of the meager thymus transplant literature shows that the previous work cannot be evaluated for our own histological studies, since none of the previous workers studied in detail the cytological changes which occur in the regenerating transplant.

In this work we used young guinea pigs, varying in age from 30 to 45 days. Both main lobes of the thymus were removed and they were immediately placed in a sterile physiological salt solu-

[1] Hassall, The microscopical anatomy of the human body in health and disease. London, 1846.

tion which was kept between 37 and 39° C. The abdominal wall was then prepared for the transplantation, and one whole thymus lobe was inserted into a pocket under the fascia. Great care was exercised to avoid bleeding into the pocket. The site of the transplant was indicated by closing the fascia with a silk ligature.

The progressive changes in the transplant were studied from 1 to 48 days after insertion, using Helly's fixation and paraffin embedding. The tissues were cut serially and stained with hematoxylin-eosin, Mallory's phosphotungstic hematoxylin, and Van Gieson.

Our work shows that the thymus gland of the guinea pig, when transplanted autoplastically undergoes a series of changes similar on the whole to those previously described in the rat.[2] These changes are characterized by degenerative phenomena which begin within a few hours after transplantation and reach their height in about two days. In the guinea pig, however, unlike the rat, excellent opportunities are afforded for the study of the degenerative and regenerative changes in the Hassall's corpuscles.

During the first 24 hours the number of corpuscles in the transplant is greatly diminished, and those remaining are markedly degenerated. They appear as small, irregularly concentric, fibrilla, practically acellular structures. At the end of the first 24 hours the transplant becomes vascularized and absorption of the debris begins. Regenerative changes set in between the 48th and 72nd hours, at the periphery of the lobules, and these are characterized by proliferation of the reticular cells which are at first spindle shaped, but as regeneration progresses these reticular cells become epitheliod, and finally polygonal in shape. Definite regeneration, with the appearance of well-formed Hassall's corpuscles, occurs about the 5th day; and the developments of these may be traced to the hypertrophy of single cells or cell groups of the reticular epithelium, which as they enlarge, push the neighboring cells aside and compress them. At the same time, the neighboring regenerated reticular epithelial cells may become hypertrophied leading to an increase in size of the Hassall's body, while the central cells undergo degeneration. Frequently one sees fusion of several corpuscles to form a large compound Hassall's body. While our sections show that Hassall's corpuscles are formed by hypertrophy of the reticular cells, there seems to be no doubt that these cor-

[2] Gottesman and Jaffe, *J. of Exp. Med.*, (in press).

puscles may also be formed, in a transplant, as a result of the development of single or multiple areas of degeneration in the centers of solid masses of reticular epithelium.

About the 8th day, the regenerating lobules, particularly at the periphery of the transplant appear like surface epithelium; the proliferated reticular cells being polygonal in shape with definite cell walls, and cement substance between the individual cells. These regenerated lobules also contain Hassall's corpuscles with areas of central degeneration, and the sections give the illusion of a malignant epithelioma.

As regeneration progresses the lobules take a lymphoid character, about the 10th day, by the appearance of small round thymic cells between the reticular epithelial cells. Concommitantly with this there is a reduction of the number and size of the Hassall's corpuscles.

By the end of the second week the lobules become differentiated into cortex and medulla. In the regenerated differentiated lobules, the Hassall's corpuscles appear either as nests of well-preserved epithelial cells with slight or no evidences of central hyalinization, or, as typical Hassall's bodies made up of concentrically placed cells, in the center of which is a mass of nuclear debris with some dense hyaline cytoplasmic or plasmic remains. The outermost cells are usually best preserved, while the remaining layers show progessively increasing keratinization.

Regenerated ducts with ciliated epithelium are occasionally seen in the thymus transplant of both the rat and the guinea pig. The ducts have developed undoubtedly from the pre-existing ducts which were present in these glands before transplantation. The development of Hassall's corpuscles from reticular epithelium is quite independent of the formation of the presence of these ducts in the transplant.

Conclusions: 1. These studies seem to bring unequivocal experimental proof that Hassall's corpuscles are derivatives of the reticular epithelium; a view originally proposed by Paulitsky and more recently elaborated by Hammar, and supported by many others on the basis of embryological and post fetal histological studies.

2. They also show that in post fetal life the formation of Hassall's corpuscles is independent of the presence of remnants of the original epithelial ducts of Remak.

2843

Inhibition of sporulation by acid fuchsin.

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Certain members of the tri-phenyl-methane series of dyes are now known to have an inhibitory effect on bacteria. This is particularly true of the basic dyes like gentian violet which are capable of impeding many of the active processes of the Gram-positive bacteria, of preventing their growth in very weak dilution, and even of killing them. These types are less potent in their action on Gram-negative bacteria. The acid dyes of this series (like acid fuchsin) are much weaker in bacteriostatic action than the basic dyes; indeed, in order to bring out their power at all, long exposure to fairly strong solutions (preferably at slight elevations of temperature) are needed. So far as acid fuchsin exhibits selective activity at all, it behaves (at least under experimental conditions in which bacteriostatic and bacteriocidal powers are combined) quite differently from the basic dyes.

In general, four effects of tri-phenyl-methane dyes on bacteria have been observed. These dyes bring moving bacteria to rest, they inhibit fission, they impede sporulation, they bring about a state of suspended animation and they kill. By choosing the dye and the organism used in the experiment, any or all of these results may, within limits, be produced at will.

The present communication has to do with the prevention of sporulation caused by exposures of *B. subtilis* to acid fuchsin. The phenomenon which has been observed appears to be similar to observations made on other dyes by Behring,[1] Roux,[2] Flexner and Noguchi,[3] and Noguchi.[4]

Plants from a 24-hour culture of *B. subtilis* were made on petri dishes, covering the entire surface of the agar, and allowed to grow 3 hours. Smears made at this time showed the growth

[1] Behring, *Ztschr. f. Hyg.*, 1889, vi, 117.

[2] Roux, *Ann. de l'Inst. Pasteur*, 1890, iv, 25.

[3] Flexner and Noguchi, *J. Exp. Med.*, 1906, viii.

[4] Noguchi, *ibid.*, 1908, x, 30.

to consist almost entirely of vegetative forms. A heavy suspension in distilled water was made of the bacteria scraped from the surface of these plates. One cc. of this suspension was placed in one tube for control. To one cc. in a second tube, 10 drops of 1 per cent aqueous solution of acid fuchsin (Grübler) were added. The two tubes were placed in a water-bath and kept at 55° C. for one hour. They were then allowed to stand at room temperature and observations made over a period of 106 days. In the control tube spores began to form promptly; at the end usually of 24 to 48 hours, smears made from this tube consisted entirely of spores. In the acid fuchsin tubes no spores whatever, or only an occasional one, could be observed. The smear from these tubes at the end of 23 days looked exactly as it did at the start of the experiment, that is to say, it consisted almost entirely of vegetative forms. That the dye had been hostile to the organism was shown by the genesistasis which it caused, for bacterial counts showed that no growth whatever had occurred. Indeed, the vegetative forms not only failed to divide, they slowly died. This was proven not only by the decreasing bacterial counts, but also by making single cell transplantations (in order to insure planting only vegetative forms without spores) at various intervals of time. On the 5th day these transplantations were all positive; that is to say, the organisms were alive but had not sporulated. On the 10th day about one half the transplantations were positive. On the 23rd day all of them were negative. The environment was therefore proven to be hostile, yet vegetative forms failed in such an environment to sporulate. This is another bit of evidence against a teleological conception of sporulation as nothing more than a protective mechanism. The process must be ultimately a chemical one. It is true that the chemical stimulus which initiates it is often hostile to the organism and the mechanism then has the appearance of protection, but a chemical stimulus may be hostile to the organism and at the same time inhibit the very sporulation which would protect bacteria against it.

2844

A new culture medium for tissues grown in vitro.

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Harrison[1] demonstrated the growth of embryonic amphibian nerve tissue in the clotted lymph obtained from an adult frog. Burrows[2] demonstrated the growth of embryonic chick tissue in blood plasma obtained from the adult hen. In most of the experiments with tissue cultures since that time the blood plasma of various animals has been utilized either alone or in combination with certain tissue extracts as a culture medium. Some investigators, notably W. H. and M. R. Lewis, have used saline solutions, such as the Locke-Lewis solution, as a culture medium.

We have recently found that the peritoneal exudate of certain animals affords a culture medium which, in the experiments thus far made, gives evidence of being superior to any medium heretofore used. In the first place, this exudate apparently affords a more natural environment for the living tissues, with a corresponding increase in cell activity and length of survival *in vitro*. Also, the method of obtaining the exudate is comparatively simple. In the case of the frog, a considerable amount (about 0.5 cc.) may usually be secured from an anesthetized animal by drawing up the fluid from near the posterior end of the peritoneal cavity with a fine sterile pipette. In the case of the guinea pig, the animal is fastened to a board, and the exudate then secured by means of a capillary pipette inserted through the ventral surface of the body wall. The tip of the pipette should just barely reach into the peritoneal cavity. Suction is applied by the operator through a rubber tube attached to the pipette. The same animal may be used repeatedly for securing the exudate, and it apparently is very little disturbed by the operation. In order to secure a more abundant exudate various substances such as blood,

* This work has been done in connection with a grant from the National Tuberculosis Association.

[1] Harrison, R. G., *J. Exp. Zool.*, 1910, ix, 787.

[2] Burrows, M. T., *J. Exp. Zool.*, 1911, x, 63.

saline solution, etc., may be injected into the peritoneal cavity a few hours before the operation.

Finally, the exudate is more easily handled after it has been secured than is blood plasma, inasmuch as elaborate precautions are not necessary to prevent its clotting. In cases of the abdominal wall puncture, where the operation is not a clean one, a blood vessel may be pierced, and as a result, varying quantities of blood will be mixed with the exudate. This causes a more rapid clotting than with the exudate alone. The appearance of the exudate varies according to the number of white blood cells present. These may be readily thrown down by centrifuging, and the clear liquid used, or the exudate may be used without centrifuging. The experiments to date show that the white cells in the medium do not prevent the activity of the tissues *in vitro*. Experiments are in progress which, it is hoped, will furnish additional evidence on the use of various exudates as culture media.

2845

Therapeutic activity of sodium thiosulphate.

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Sodium thiosulphate was introduced into therapy several years ago by one of us,[1] in cooperation with Dennie and McBride, as a means of relieving certain types of intoxication, such as the metallic toxemias. The drug, simple as it may seem, has fully met the vital test of yielding prompt relief when administered soon after the development of the toxic symptoms.

Our interest has led us now for several years to a further study of its *modus operandi*. The authors[2] have previously reported on

[1] McBride, W. L., and Dennie, C. C., Treatment of Arsphenamine Dermatitis and Certain other Metallic Poisonings. *Arch. f. Derm. u. Syphil.*, 1923, i, 63.

[2] Groehl, M. R., and Myers, C. N., Sodium Thiosulphate in the Treatment of Dermatitis and Jaundice as a Result of Metallic Intoxication. *Therap. Gaz.*, 1924, x, 691.

the excretion of arsenic in cases of arsenic dermatitis. The action of sodium thiosulphate is least marked in cases of inorganic arsenic poisoning; next in order, it is effective after the pentavalent organic preparations; and the greatest efficiency is reached with the trivalent group represented by the arsphenamines. The activity of sodium thiosulphate cannot be judged by the excretion of arsenites. It has been pointed out by Mueller and Myers[3] that the skin and the liver are the two main organs involved in intoxication, and on this basis sodium thiosulphate serves as a protective drug in respect to the autonomic nervous system.

The present report offers clinical and experimental evidence in favor of the efficacy of freshly prepared sodium thiosulphate in hastening the excretion of arsenic either in its inorganic or in its organic form. Furthermore, sodium thiosulphate does not disturb the therapeutic action of the arsphenamine compound in its action on Trypanosome equiperdum. Prompt results have been noted by leading syphilologists in connection with dermatitis and jaundice following the use of the arsphenamine group. Some clinical data have recently been made available through the investigations of H. H. Dale, of the British Medical Research Committee.[4]

Table I shows the effect of the simultaneous use of salvarsan and sodium thiosulphate, together with the control experiment. Table II shows the action of sodium thiosulphate on the excretion of arsenic in the urine. The clinical history of the case is as follows:*

Male, aged 39, four years ago began to show groups of vesicles on an erythematous base. The vesicles were exceedingly itchy. Diagnosed as *Dermatitis herpetiformis*. These developed the typical pigmentation and scarring of that disease. Two years ago, began taking arsenic in the form of Asiatic pills—nine a day for four months; received solution of potassium arsenite (Fowler's solution) in gradually increasing doses.

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[3] Mueller, E. F., and Myers, C. N., The Effect on the Involuntary Nervous System of Arsenicals and the Salvarsan Group. PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 474.

[4] Harrison, L. W., Effect of Sodium Thiosulphate on the Therapeutic Power of Arsenobenzol Compounds. *Lancet*, 1925, xxii, 1161.

* From the Service of Dr. Throne and Dr. Clark, New York Skin and Cancer Hospital.

TABLE I.

Rat No.	Wgt.	Dose mgm. per kg.	Trypanosome count at time of treatment.	1st day	2nd day	3rd day	5th day	7th day	8th day	9th day	10th day
G-87 (in 20% $Na_2S_2O_3$) G-87 64 mgm. in 40 cc. water. $Na_2S_2O_3$ 8 grams											
5793	166	3.5	136,000	—	+ — —		D				
5794	168	3.5	164,000	—	+ — —		+	D			
5795	162	3.5	116,000	1000	—		—	D			—
5796	165	5.3	108,000	—	—		—				+ + + +
5797	160	5.3	172,000	—	—		—				D
5798	167	5.3	112,000	—	—		—				—
5799	161	8.	136,000	—	—		—				—
5800	171	8.	176,000	—	—		D				
5801	164	8.	124,000	—	—		—		—		
5802	159	12.	188,000	D							
5803	167	12.	132,000	—	—		D				
5804	160	12.	156,000	—	—		—		—		
Minimum effective dose = 3.5.											
SALVARSAN G-87 160 mgm. in 100 cc. water.											
5805	162	3.5	164,000	1000	+		D				
5806	167	3.5	136,000	—	—		—		D		
5807	162	5.3	68,000	—	—		—		—		
5808	168	5.3	100,000	—	—		—		—		
5809	156	8.	120,000	—	—		—		—		
5810	157	8.	168,000	—	—		—		—		
Minimum effective dose = 3.5.											

TABLE II.—URINE SPECIMENS.

Spec. No.	Date	Period	Excreted cc.	Sp. gr.	Moist wt. of spec. gm.	Dry wt. of spec. gm.	Per cent solids	As in mg. per 100 gm. spec.		Notes.
								Moist	Dry	
8569	9/25/24	24 hrs.		1.019	35.4730	0.9395	2.64	0.002	0.10	Sodium thio. 15 gr. daily
8566	9/26/24 A.M.			1.17	36.2010	0.8270	2.28	0.01	0.48	
8570	9/26/24 P.M.	24 "		1.010	24.0012	0.3647	1.52	0.03	2.19	
8571	9/29/24	24 "		1.019	35.7780	1.1400	3.18	0.08	2.63	
8572	10/ 2/24			1.015	34.5480	0.5540	1.60	0.005	0.36	
8579	10/ 6/24	24 "	1800	1.020	30.8457	0.6676	2.12	trace	trace	No sod. thio. on this day
8580	10/ 8/24 A.M.		2500	1.019	22.1320	0.5800	1.80	0.003	0.17	
8578	10/ 8/24 P.M.		2500	1.017	33.4710	0.5810	1.73	0.005	0.34	
8581	10/10/24		1000	1.021	26.1375	0.6575	2.33	0.01	0.61	
8585	10/16/24	24 "	1000	1.023	31.5030	1.2470	3.96	0.005	0.12	
8582	10/17/24 A.M.	24 "	1000	1.020	35.7780	0.0710	2.99	0.006	0.19	No sod. thio. on this day
8583	10/17/24 P.M.		1000	1.022	38.6333	1.5723	3.97	0.05	1.27	
8584	10/21/24	24 "	1250	1.017	39.3532	1.0842	2.75	0.002	0.05	No sod. thio. on this day
8587	10/27/24 A.M.				38.2150	1.7850	4.66	0.003	0.05	No sod. thio. on this day
8592	10/27/24 P.M.			1.026	27.0376	1.4176	5.26	0	0	
8593	10/31/24	24 "	1400	1.019	24.8400	0.7700	3.09	trace	trace	
8591	11/ 3/24	24 "	1000	1.015	28.0753	0.6703	2.37	0	0	1 gm. sod. thio in 10 cc. water intravenously daily
8593	11/ 4/24	24 "		1.020	43.5071	1.5851	3.64	0.06	1.51	
8596	11/ 7/24	24 "	700	1.022	46.9760	1.8720	3.98	trace	trace	No sod. thio. on this day
8597	11/ 9/24	24 "		1.023	48.1793	1.6043	3.33	0.002	0.06	
8598	11/11/24	24 "		1.022	49.9590	2.1020	4.26	0.04	0.95	
8603	11/13/24	24 "	850	1.025	25.6400	1.1400	4.44	0	0	No sod. thio. on this day
8604	11/15/24	24 "	800	1.020	27.5377	0.9587	3.44	0.005	0.16	
8670	12/ 2/24		800	1.031	36.0415	1.9420	5.38	0.004	0.08	No sod. thio. on this day
8671	12/ 4/24		900	1.032	39.9013	1.9798	4.99	0.004	0.08	No sod. thio. on this day
8672	12/ 6/24		950	1.027	33.8245	1.5635	4.67	0.11	2.29	
8673	12/ 8/24		600	1.033	39.6360	2.1005	5.30	0.10	1.90	
8676	1/13/25			1.010	39.1620	0.6870	1.76	trace	trace	No sod. thio. on this day
Calamine lotion			20		23.5788	7.1003	30.11	0.25	0.84	

Calamine lotion used on skin. The high arsenic content is to be noted.

After entering the hospital a biopsy was done and histological examination made. The patient was immediately given injections of sodium thiosulphate intravenously, 1 gm. every other day, and 15 grains (0.97 gm.) of thiosulphate three times a day after meals. The intestinal bleeding stopped at once, and under continued injections the acute dermatitis of his head, hands and feet slowly subsided, and the peculiar pigmentation with lichenoid papules scattered through it—particularly on the body and on the back of the neck—showed improvement.

Summary. Sodium thiosulphate shows no deleterious action on the trypanocidal activity of the salvarsan groups when the two drugs are given simultaneously. These results are corroborated by clinical observations of other investigators. Sodium thiosulphate plays an important part in the rate of excretion and also the clinical symptoms following an intoxication due to arsenic in (a) inorganic state, (b) pentavalent organic state, and (c) the organic trivalent arsenicals.

Clinical study showed that when the injections of thiosulphate were stopped and the arsenic output in the urine dropped down to about 0.003 to 0.004 mg., the eruption on the head, hands and feet and the peculiar lichenoid eruption on the back, chest, arms and legs regularly became worse. With the renewed administration of the thiosulphate, and when the arsenic output came up to about one decigram of arsenic for each 100 gm. of moist specimen, the eruption regularly improved.

2846

Hyperglycaemia following experimental cholecystitis.

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A series of fifteen experiments were conducted as follows: A presumably normal mongrel dog was prepared for laparotomy, by administration of morphia and ether, shaving, and cleansing of the abdominal field for operation. Under sterile conditions the abdomen was opened, the gallbladder was identified and incised.

Summary of Experiments on Pancreatitis Secondary to Cholecystitis.

Exp. No.	Duration in days.	Mg. blood sugar per 100 cc. blood		Pathological Findings
		Preoperation	Postoperation	
3	17	88	?	—
5	60	96	170 120 260 160 160 130 180	Cholecystitis Lymphadenitis of biliary, pancreatis, and coeliac nodes
6	60	100	150 140 140 165 175 150 175	Cholecystitis Liver congested Lymphadenitis Pancreas thickened
8	37	88	146 100 140 148 166	Cholecystitis Lymphadenitis Pancreas thickened
11	2	?	?	—
17	0	134	—	—
18	3	260	?	Death from infection
19	39	116	126 105 90 170 136	Cholecystitis Lymphadenitis Abscess of pancreas Capsular and interlobular infiltration; congestion; hyalinization of islands
20	35	136	120 195 192	Cholecystitis Lymphadenitis Pancreas thickened
21	1	156	?	Death from infection
24	32	240	100 164 160	—
35	6	126	92	Cholecystitis Cloudy swelling of pancreas Fatty degeneration liver
37	10	110	108	Liver congested; Cholecystitis; lymphadenitis; pancreatitis. Necrosis of acinar cells.

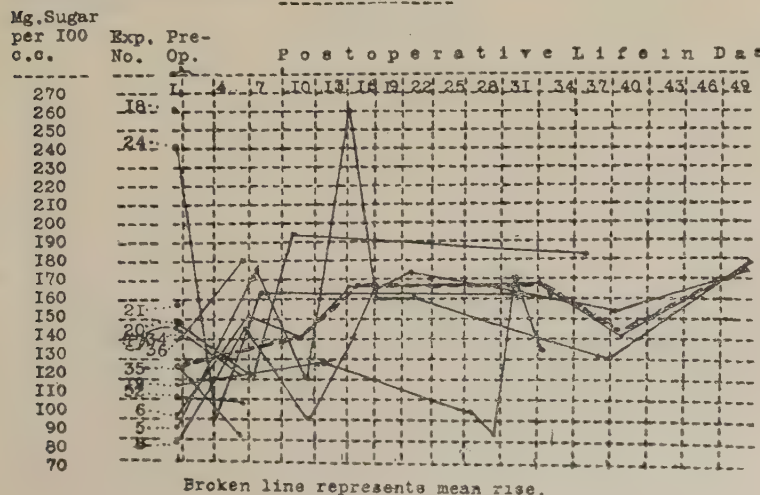
A glass bead containing faecal matter and bacteria was dropped into the bladder, the bladder was closed with suture, and left attached within the plane of closure of the abdominal wound. After such an operation the animal was placed in a recovery cage and fed water, fluids, and solid material according to the return of the dog's appetite. The diets of the animals fully recovered from operation consisted of meat scrap from the tables of the Hospital dining-room. The blood sugars were examined in each such animal before operation and at intervals of three to five days after operation. After an interval of one to sixty days, each of the experiments was terminated by dispatching the animal and by determining the pathological changes that had taken place within the dog's abdomen.

The particular blood sugar findings and pathologic changes are summarized on the accompanying chart. The fluctuations in blood sugar content in relation to the mean sugar content of the total series studied appears on comparison of the lines on the diagram below.

Glycosurias were noted in the animals examined.

It is worthy of special attention that the animals showing rises in the blood sugar after operation were animals in which pathologic changes were taking place about the gallbladder, in the lymphatics of the bile ducts, liver, and pancreas, or in the pan-

DIAGRAM REPRESENTING BLOOD SUGAR TRACINGS IN DOGS BEFORE AND AFTER PRODUCTION OF EXPERIMENTAL CHOLECYSTITIS



Broken line represents mean rise.

creas itself. Two animals with a distinct fall in the blood sugar after operation gave no evidence of lymphadenitis. The mean rise for ten observations was from 125 to 140 mg. per 100 cc. blood for the first 12 days, or, from 125 to 165 mg. for the first month after the introduction of infectious material in the gallbladder. Experiment No. 19 with an abscess of the head of the pancreas was narcotized on the 32nd day, during a rise in sugar, to determine the possible effects of anesthesia upon the blood sugar. It was observed in this instance that the percentage fell from 170 to 135 mg. in the succeeding three days. It is felt that the rise in the blood sugar in these experimental animals has been due not to anesthesia, not to diet, not to confinement of the laboratory animals, but to morphological changes in the pancreas consequent upon infection lymphatic-borne from the gallbladder and liver.

The frequent association of diabetes or hyperglycaemia with cholecystitis, lymphadenitis and pancreatic thickening in the human patient, as met with in the surgical ward of Bellevue Hospital, has led to the suspicion that the diabetic state may be in part at least of infectious origin. The above experiments were undertaken to determine whether infection arising in the gallbladder and spread by the lymphatics gives rise to hyperglycaemia.

2847

Differentiation of B. aerogenes and B. coli of non-fecal origin from B. coli of fecal origin.

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Koser [1, 2, 3] has demonstrated with the "colon bacilli" that *B. aerogenes* and *B. coli* isolated from soils utilize citrates as a a source of carbon and that *B. coli* of fecal origin does not use it.

[1] Koser, S. A., *Abs. Bacteriol.*, 1924, viii, 6.
[2] Koser, Stewart A., *J. Bacteriol.*, 1923, viii, 493.
[3] Koser, Stewart A., *J. Bacteriol.*, 1924, ix, 59.

One of us was interested in isolating iron precipitating bacteria. Harder's[4] medium which consisted of K_2HPO_4 0.5 gm., $MgSO_4$ 0.5 gm., $(NH_4)_2SO_4$, 0.5 gm., $CaCl_2$, 0.2 gm., $NaNO_3$, 0.5 gm., Ferric Ammonium citrate 10 gm., H_2O, 1000 gm., Agar 18 gm., was used. The medium is pale yellow in color; when the iron precipitating bacteria grow on this they produce a vivid heavy iron rust streak. It is possible that these bacteria use the citrate and mechanically precipitate the red iron compound. It was thought of interest to try the "colon bacilli" on this medium and compare it with Koser's medium, which consists of NaCl, 5 gm., $MgSo_4$, 0.2 gm., (NH_4) H_2PO_4, 1 gm., K_2HPO_4, 1 gm., and sodium citrate 2 gm., in 1000 gm. H_2O.

Ninety-one strains of the "colon bacilli" were isolated from sewage, (and in addition a *B. coli* of fecal origin, a *B. coli* of non-fecal origin and a *B. aerogenes* kindly supplied by Dr. Koser) were used. These were streaked on the Harder medium and inoculated into Koser's medium. The Voges-Proskauer test was also carried out. The incubation was 2 to 5 days at 37½° C. The following results were obtained:

45 (*B. coli,* Voges-Proskauer negative) did not darken the Harder medium nor produce turbidity in the Koser medium.

47 (*B. aerogenes,* Voges-Proskauer positive) darkened the Harder medium and produced turbidity in the Koser medium.

2 (*B. coli,* Voges-Proskauer negative, Intermediate) darkened the Harder medium and produced turbidity in the Koser medium.

The *B. aerogenes* and the *B. coli* of non-fecal origin produced a vivid red iron rust growth on the Harder medium. This was very pronounced and easier to read than turbidity in the liquid medium.

The Harder liquid medium (without the agar) was next tried. *B. aerogenes* and *B. coli* of non-fecal origin produced a dark brown flocculent precipitate. This was, however, delayed for several days. The reaction was not as fast or as intense as on the Harder solid medium.

In addition to this work the sodium citrate was replaced in Koser's medium by the ferric ammonium citrate and agar added. This new medium was compared with Koser's liquid medium. Thirty-one cultures were tested with the following results:

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[4] Harder, Edmund Cecil, United States Geological Survey, 1919, Professional Paper, 113.

15 (*B. coli*) did not grow on the solid medium nor did they produce any turbidity in the liquid medium.

15 (*B. aerogenes*) darkened the solid medium and produced a turbidity in the liquid medium.

2 (*B. coli* intermediate) darkened the solid medium and produced a turbidity in the liquid medium.

Comparing the Koser solid medium in which ferric ammonium citrate was substituted for the sodium citrate with the Harder solid medium, it was evident that on the latter the growth and intensity of the red color was much better.

The Harder solid medium offers an easy and brilliant method for the differentiation of *B. aerogenes* and *B. coli* of non-fecal origin from *B. coli* of fecal origin.

2848

The specific fraction of alcohol soluble specific substance of the tubercle bacillus.

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In a previous paper[1] it was shown that the specific substance of the tubercle bacillus, soluble in lipoid solvents, can be prepared from ether, ethyl and methyl alcohol extracts in a similar grade of purity; the potency and the chemical composition of the preparation is very similar. With the further purification of the product as described in the paper mentioned, and with the separation of it into different fractions by precipitation from warm methyl alcohol, from acetic acid and from chloroform solution, etc., we never obtained markedly more potent preparations. The bulk of the purified substance, which is supposed to contain a large percentage of the specific substance, is composed of fatty acids. Besides these, it contains quite a large amount of P. (2.6 per cent), home reducing sugar (in one case only 4 per cent), and it contains only traces of N. (0.3 per cent).

[1] Dienes, L., and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

During further work with this preparation a fortunate chance helped us to separate the antigenically active part of the preparation from the lipoidal part. Quite a large amount of the specific substance was prepared from an alcohol extract of tubercle bacilli as formerly described. The preparation showing the maximum potency was dissolved in ether, and the ether solution was washed with diluted HCl for the elimination of the substances containing N, then several times with distilled water, and kept for 24 hours over anhydrous Na_2SO_4. The perfectly clear solution, which after this procedure has shown unchanged potency for several weeks, first developed a turbidity, then deposited a heavy precipitate and simultaneously with the appearance of the precipitation its potency decreased. The container during this time was opened several times, but after every opening filled with CO_2. The precipitate was freely souble in methyl alcohol and in water, and in the complement fixation test it was

TABLE I.

	Potency in complement fixation Unit	Solubility		Chemical Composition
Specific substance in the original ether solution.	0.0002 mg.	Freely soluble in ether, about 2 per cent in alcohol; is precipitated with aceton. The aceton contains only traces of the specific substance.	It is extracted from a watery emulsion with ether and it is retained by a paper pulp filter.	About 65% fatty acids 2.6% P, and 0.3% N.
Aceton soluble fraction after splitting.	0.00004 mg.	Non-soluble in ether; freely soluble in alcohol, aceton, water.	It is not extracted from a watery emulsion and is not retained by paper pulp; not even if the watery emulsion is made from a mixture of the specific substance and a crude alcohol extract.	15% fatty acids(?) 7.2% P and 0.5% N.
Ether soluble fraction after splitting.	0.02 mg.	Freely soluble in ether, alcohol and aceton.		80% fatty acids(?) 1% P.

5 times as potent as the formerly most potent preparations. By precipitation with aceton the specific substance went into the aceton solution. The aceton solution was evaporated and extracted first with ether, then again with aceton. In Table I are described some properties of the specific substance before and after separation from the lipoids, and of the remaining fatty substances after the separation. In addition the specific substance in the state of the highest purity gave 17 per cent carbohydrates after hydrolysis, and by the combustion (according to Pregl) has shown 8.85 per cent H and 36.0 per cent C. The significance of the carbohydrates is not established, because the aceton non-soluble residue of the precipitate contained 33 per cent carbohydrates without showing any specific activity. It may be that the fatty acids present in the specific substance (the ether soluble substance after hydrolysis) are the result of a contamination with the ether soluble substance.

The changes in the properties of the specific substance and of the remaining solution after their separation are so thorough that we must suppose that it is the result of a chemical splitting. The alcohol soluble specific substance of the tubercle bacillus, as we obtain it in our extracts, consists according to this, of a non-lipoidal specific part containing no, or at least very little, chemically connected with lipoid substances. It may be that the connection with the lipoid substances is the cause of a weak antigenic effect *in vivo,* although the antibody formation has only been observed with non-purified extracts.

In case the specific substance in the original extracts forms a chemical compound with the lipoids we must suppose the existence of a new class of lipoid substances which contains P and no N and the yet undefined specific substance in the molecule.

If the substance described in this paper is the same as the substance found by Laidlow and Dudley[1, 2] cannot be decided. The solubility of the two substances seems to be different.

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[1] Dienes and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

[2] Laidlow, P. P., and Dudley, H. W., *J. Exp. Path.*, 1925, vi, 197.

2849

The action of the lytic principle on capsulated bacteria.

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Since capsulated bacteria, or bacteria in the capsulated state, are known to be more resistant to the action of harmful agents than are organisms without capsules, the question of the resistance of typically capsulated bacteria to the lytic principle is of interest.

The cultures employed in the experiments to be reported were as follows: (1) A laboratory stock strain of the Friedländer pneumo-bacillus; (2) a strain of the same freshly isolated from the root canal of an infected tooth; (3) a laboratory stock strain of *B. ozenæ;* (4) a laboratory stock strain of *B. rhinoscleromatis.* The culture medium was a beef infusion broth and beef infusion agar, pH = 7.8. From each of these cultures in the presence of sewage-contaminated river water an active lytic agent was isolated by the usual methods of alternate feeding and filtration.

The maximum dilution of the lytic filtrates which gave inhibition of the homologous culture (one loop in broth) was about the same as in the case of the Shiga dysentery lytic principle, namely, 10^{-8}. Beyond this point the titer could not be raised and sometimes it did not attain this degree.

The lytic filtrate developed from the laboratory strain of Friedländer was effective in causing the inhibition and lysis of the strain from the infected tooth, but not against *B. ozenæ* or *B. rhinoscleromatis.* In a similar manner, the lytic filtrate developed against the tooth strain of Friedländer was active against the laboratory strain but not against the others. The lytic filtrates from *B. ozenæ* and *B. rhinoscleromatis* were not reciprocal in action; neither did they influence the Friedländer strains.

In the case of all the lytic filtrates acting, in proper dilution, on their homologous strains on agar surfaces, lytic areas were formed. These were usually smaller than the typical Shiga lytic areas and showed a tendency toward irregular shape rather than round. In addition, and particularly in the case of Friedländer,

there was some overgrowth of the lytic spots by the surrounding culture. For this reason the cultures sometimes came to resemble normal cultures in respect to their freedom from erosions.

Both of the Friedländer strains mentioned above were dissociated into the pure S- and R-components by the usual methods. The S-type much resembled the mother culture while the R-type was quite different. The original cultures were mucoid, both in broth and on agar, and stained preparations from both showed heavy capsules. After dissociation, the S-type showed similar characteristics. The R-type, on the other hand, grew in infusion broth and on agar with no sign of mucoid characteristics and, in stained preparations showed no capsules. In young, agar slant cultures, while the S-type was heavy and opaque, the R-type was thin and translucent. After a few days growth, however, the two types came to resemble each other superficially, but the difference with reference to capsules remained; the S-type showed good capsules while the R-type showed none. Up to the present time the R-type has indicated no tendency to assume capsule formation.

Against the S- and the R-types of both strains of the Friedländer bacilli active lytic filtrates were developed from sewage contaminated water. The lytic agent developed as readily against the capsulated S-type as against the non-capsulated R-type, and both filtrates attained about the same power of inhibition and lysis on the homologous culture. Moreover, the lytic filtrate developed against the S-type produced both inhibition and lysis of the R-type and vice-versa. Both filtrates showed the same action on the original stock cultures of Friedländer and in about the same degree. Comparing the action of the Friedländer filtrates with that of the typical d'Herelle bacteriophage on Shiga cultures the only noteworthy difference observed was a slower action of the Friedländer filtrates.

When a few loops of concentrated Friedländer lytic agent were applied to the surface of agar slants and the slants then streaked with broth culture (either R- or S-type) no growth took place for a day or more. Eventually, however, the customary secondary growth appeared in the form of small colonies of the correlated resistant strain. These colonies (SR- and RR-types) differed from the original Friedländer colonies and also from the S- and the R-colonies. Moreover, the SR-colonies differed from

the RR-colonies. Neither the SR- nor the RR-cultures were susceptible to inhibition or lysis by the original, the S- or the R-lytic filtrates. Thus, by the combined action of dissociation and the lytic principle, the Friedländer cultures were split into at least four sub-types. The biological characteristics of these have not yet been fully studied.

In conclusion it may be said that, at least in the case of the Friedländer bacillus, as also in the instance of *B. ozenæ* and *B. rhisclerom atis,* there is no evidence that capsule formation offers any hindrance to the inhibitive or the lytic action of the bacteriophage. This conclusion confirms the observation of Paul Caublot[1] who has also recorded the existence of a lytic agent for the pneumo-bacillus.

2850

Studies on the state of the serum calcium.

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(Introduced by H. T. Karsner).

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In low phosphorus rickets, the animal is unable adequately to utilize its calcium in spite of the fact that the blood contains a normal amount of calcium. In low calcium rickets the total calcium of the blood is low but, as shown by the addition of the fat soluble organic factor, the diet supplies enough calcium to provide for a normal amount in the blood. These facts make it seem of importance to determine the ratio of diffusible and colloidal serum calcium in the blood in the two conditions.

Three litters of rabbits were used, as indicated in Table I. The diffusible serum calcium was separated by negative pressure filtration through a collodion membrane as described by Moritz.[1] The rabbits were fed through a stomach tube twice daily throughout

[1] *Compt. rend. Soc. de Biol.*, 1924, xc, 622.

[1] Moritz, Alan R., The Effect of Ultra-Violet Irradiation on the State of the Serum Calcium. *J. Biol. Chem.*, 1925, lxiv, 81.

TABLE I.
The State of the Serum Calcium in Experimental Rickets in Rabbits.

Litter	Diet	Animal No.	Total Ca.	Diff. Ca.	% Diff. Ca.	In. Phos.	Rickets
I	—P—A	1075	13.5	7.31	54	3.25	Severe
		1076	13.0	6.45	50	3.1	Severe
		1077	11.55	6.06	52	3.1	Severe
	—P+CLO	1073	11.9	6.54	55	8.9	None
		1074	10.5	6.06	58	8.17	None
II	—P—A	1082	13.7	8.62	63	3.8	Slight
		1088	13.08	7.39	52	3.8	Moderate
		1089	10.85	6.00	51	3.6	Osteoporosis
	—P+CLO	1083	13.47	7.90	59	10.3	None
		1085	13.23	8.00	61	8.6	None
		1086	12.54	8.23	66	6.1	None
III	—Ca—A	1161	7.7	5.6	72	Not determined	Osteoporosis
		1162	8.2	4.9	59	”	Osteoporosis
		1163	8.8	5.1	55	”	Osteoporosis
	—Ca+CLO	1160	12.0	7.9	65	”	Osteoporosis

the course of the experiment as in the studies of Goldblatt and Moritz.[2]

Litter I and Litter II were placed on a diet deficient in phosphorus and the fat soluble organic factor. Two animals of Litter I and three animals of Litter II served as controls and received five drops of cod liver oil daily. At the end of thirty days all animals of litter I were killed. The three experimental animals had developed severe rickets while the controls showed no evidence of rickets.

Litter II were killed at the end of eighteen days. Two of the experimental animals had developed moderate rickets while the third showed only severe osteoporosis. The controls showed no evidence of rickets.

Litter III received for 23 days a diet deficient in calcium as well as the fat soluble organic factor. One animal served as a control and received five drops of cod liver oil daily. Although these animals received a diet which is rickets-producing in a rat, it was found that a severe degree of osteoporosis masked any rachitic changes that might have been present.

Table I shows that all of the experimental animals of litters I and II showed a marked diminution of the inorganic phosphorus of the serum as compared with the controls, while there was no significant alteration in the amount of the serum calcium. The per cent of the diffusible serum calcium in experimental and control animals did not differ significantly. In litter III the serum calcium of the three experimental animals was greatly reduced as compared with the control. However, there was no significant difference in the per cent of the diffusible serum calcium in the experimental and control animals.

In low phosphorus rickets of rabbits, where the total calcium of the serum is normal, the percentile ratio of diffusible and colloidal calcium remains essentially undisturbed. In rabbits fed on low calcium diet deficient in the fat soluble organic factor the percentile ratio of diffusible and colloidal calcium also remains essentially normal in spite of a great reduction of the total serum calcium.

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[2] Goldblatt, Harry, and Moritz, Alan R., Experimental Rickets in Rabbits. *J. Exp. Med.*, 1925, xlii, 499.

2851

Some changes in the acid base equilibrium of the body caused by hemorrhage.

MARY A. BENNETT (Introduced by D. Wright Wilson).

[*From the Laboratory of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadephia, Pa.*]

Hemorrhages of one-third to one-half of the total blood volume in dogs produced the following changes. The sudden fall in alkaline reserve (the total CO_2 content of arterial blood was determined in the present investigation) which has been noted by various investigators, was associated with a decrease in pH of the blood, this decrease amounting to as much as .10 pH. On the day following the hemorrhage the total CO_2 was normal, or above. At this time the blood was slightly more alkaline than normal, the increase varying from .03 to .09 pH.

The CO_2 tension of the arterial blood (calculated from the pH and total CO_2 of blood) fell immediately after hemorrhage. Several hours later it was low, normal or high. The next day it was always low. The hematocrit fell rapidly, slowly, or not at all, immediately after hemorrhage. A minimum value was obtained on the day following the hemorrhage. It appears that the increased alkalinity of the blood is associated with the restoration of blood volume.

2852

Cullen's colorimetric method for the determination of the pH of blood plasma.

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In experiments on hemorrhage, Cullen's[1] colorimetric method for the determination of the pH of plasma was found unreliable even for comparative studies. The correction of 0.35 for dogs' plasma, necessary to convert the colorimetric readings of pH at 20°, to the electrometric pH at 38°, varied especially after hemorrhage, from 0.42 to 0.19.

Hastings and Sendroy[2] stated that Cullen's correction disappears if the colorimetric determinations are made at 38° instead of 20°. In a single experiment where this procedure was also used, the corrections became less, but did not disappear, and the variations noted above persisted.

2853

Comparison of the pH of serum and plasma.

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While the experiments reported above were being carried out, Hirsch[1] stated that serum is more acid than plasma. In a later paper[2], however he reported data throwing doubt on the accuracy of his previous conclusion. In order to determine whether there was any difference between the pH of serum and of plasma of dog's blood, electrometric and colorimetric pH determinations were done on both, Cullen's[3] colorimetric method was used. We

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1 Cullen, Glenn E., *J. Biol. Chem.*, 1922, lii, 501.

2 Hastings, A. Baird, and Sendroy, Jr., Julius, *J. Biol. Chem.*, 1924, lxi, 695.

1 Hirsch, E. F., *J. Biol. Chem.*, 1924, lxi, 795.

2 Hirsch, E. F., *J. Biol. Chem.*, 1925, lxiii, 55.

3 Cullen, Glenn E., *J. Biol. Chem.*, 1922, lii, 501.

found no difference between the serum and plasma pH, electrometrically determined. The correction in Cullen's method, however, is slightly greater for serum than for plasma as stated by Cullen.

2854

The skin response of rabbits to non-hemolytic streptococci.

C. L. DERICK and C. H. ANDREWES (Introduced by H. F. Swift).

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A study of the skin reactions in rabbits which were induced by intra-dermal injection of non-hemolytic streptococci demonstrated an unusual phenomenon. Following the injection of these microorganisms a local reaction took place which reached its maximum within 24 to 36 hours. The lesion then diminished until the eighth day, when an exacerbation occurred which at times approached the original lesion in intensity. This persisted for about 2 days and disappeared slowly. The late lesion is referred to as the "secondary reaction."

This reaction was first encountered while studying a green streptococcus isolated from a rheumatic subcutaneous fibroid nodule; with it definite secondary reactions were induced in 50 per cent of normal adult rabbits and doubtful reactions in an additional 13 per cent. On further extending the strains of green streptococci studied, the reaction was found to be incited by 5 out of 10 strains from nodules, blood or heart of rheumatic fever patients; by 1 out of 4 strains from the blood of subacute bacterial endocarditis patients; by 1 strain from the urine of a nephritic patient and by 1 anomalous strain isolated from a rheumatic nodule. It never followed the injection of green streptococci from normal throats, nor of hemolytic streptococci, staphylococci, *micrococcus catarrhalis, B. coli,* and *B. influenza.*

The material used for intra-cutaneous injection consisted of the centrifuged sediment from 5 cc. of an 18-hour growth of the

bacteria in blood broth. It was subsequently found that the reaction could be obtained with heat killed organisms.

Lesions were excised daily and studied bacteriologically and histologically. In the early stages an abundant growth of streptococci was obtained, but the number of viable organisms progressively diminished until the 7th day, when none was present, although many could still be seen in the smears. At the time of the secondary reaction only occasional contaminants were found. Histologically the primary reaction always consisted of a well circumscribed collection of cells, mostly polymorphonuclears, and bacteria, with edema of the surrounding tissue. Aside from more marked congestion and edema, there was nothing of importance to distinguish a secondary lesion from a primary one with the exception of the presence of a few macrophages in the outer cellular zone, which made their appearance about the 6th day. This secondary lesion rarely broke down and discharged pus.

By means of previous inoculation with any type of streptococcus within a period of nine weeks it was possible to prevent the secondary reaction following inoculation with a suitable strain. This protection did not exist after the ninth week. Protection was afforded not only by streptococci, but occasionally by pneumococci. Additional experiments with the latter organism are now under way. The secondary reaction was neither caused by nor prevented by nucleoprotein, soluble precipitable substance, or filtrates from an active strain.

In attempting to explain this phenomenon the possibility of renewed bacterial growth at the time of the secondary reaction was eliminated by using killed cultures and also by the inability to recover the original organism at this time. It would appear then to be caused by one of the following substances: (1) a product of the streptococci; (2) a product of the animal host; (3) a product of the interaction of streptococci and animal host.

The first is unlikely unless it takes about 9 days for the organisms to be broken down so that a toxic product is liberated. The second is hardly plausible, since such a hypothetical substance is called into action by only a limited number of bacterial strains. The third possibility has more claim for consideration.

The secondary reaction has certain analogies with serum sickness in man and the Arthus phenomenon in rabbits. To accept such an hypothesis it must be assumed that the injected strepto-

cocci or some antigenic product of them exists at the site of inoculation until the appearance of antibodies about the 8th or 9th day when there is a union of antibody and antigen; this product then acts as an irritant and excites the secondary reaction. In attempting to prove this many of the experiments of Opie[1] were repeated.

(1) Injection of streptococci into an immune animal.

(2) Injection of antibodies (immune serum) into an animal in whose tissues streptococcal antigen was known to exist.

(3) Injection of *in vitro* mixtures of antigen and antibodies, *i. e.*, homologous immune serum.

(4) Attempt to find some correspondence between the occurrence of the secondary reaction and the presence of antibodies in the body fluids at that period, *i. e.*, agglutinins, precipitins.

None of the experiments place the secondary reaction in a class with the Arthus phenomenon. As yet the causative factor or factors for the secondary reaction have not been elucidated, but further experiments are in progress toward this end.

[1] Opie, E. L., *J. Immunol.*, 1924, ix, 231.

Western New York Branch

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2855

Comparable cell changes in central nervous system in cretinism, parathyriod tetany and fatigue.

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Representative sections from the motor cortex, thalamus, midbrain, cerebellum, medulla oblongata and cervical enlargement of the spinal cord, from each of five cretin lambs and two cretin goats were studied microscopically. A modification of Nissl's method was employed. Comparable preparations from three normal animals of the same sex and age (twins of three experimental animals) furnished the control material. The primary purpose of this series of investigations was to determine the nature and course of the nerve cell changes in the central nervous system following the experimental removal of the thyroid glands, and to attempt to correlate these findings with the well known symptoms of athyreosis, many of which are referable to central origin.

It was further desired to compare the nerve cell pictures presented in these cases with those from pathological conditions of quite different etiology and symptomatology in order to determine more fully the nature and degree of specificity of the reaction. To this end the affection of parathyroid tetany was selected, almost the antithesis of the first named condition. If chromatolysis follows in the latter event where neuromuscular activity is frequently at its maximum, should we expect to find a piling up of the extranuclear chromatin in the former where the symptoms are so strikingly opposite in character? Nissl preparations were made from the brains and spinal cords of thirteen

dogs in which tetany had been induced experimentally. These were taken from the same levels as described above for the cretin animals.

For further analysis the comparison was extended to cell changes in the central nervous system as a result of functional activity. Fatigue was induced in sixteen white rats by forcing them to swim in a tank of warm water (body temperature) for periods varying from fifteen minutes to six hours.

Such a comparative study has revealed, in each case, a definite but somewhat variable chromatolysis. No marked variations in the nature and course of the affection were found to follow these conditions so strikingly opposite in character. From the material at hand, the type of reaction was clearly quite independent of the seat of action or nature of the opposing condition. It was a question of quantitative rather than qualitative variation from the normal. Cellular alterations representative of steps in the course of the reaction were readily recognized. The severity of the visible symptoms (tetany, cretinism and exhaustion) was the constant guide in selecting these types. To demonstrate the consecutive stages found and the correctness of the conclusions drawn from them, twenty-four slides were shown.

Early in the chromatolytic reaction, as measured by the severity of the external symptoms, the cells stain more deeply than normal. This intense staining is found in the nucleus as well as in the cell body, but usually in a less marked degree, and cannot be entirely accounted for by the accompanying shrinkage which is almost invariably associated with it. There is either an actual increase in the amount of extranuclear chromatin or an increase in the affinity of this substance for the basic stains. The intensity of the stain is too great to be explained otherwise. At an early stage a part of this substance is dissolved in the cell fluids, even within the nucleus, rendering all outlines extremely indistinct at times. The maximal expression of this condition is reached at a relatively early stage in the process. This is evidenced by the fact that it invariably appears after thirty minutes of activity, in mild cases of cretinism and in mild parathyroid tetany. It is soon followed, however, by a progressive fragmentation and dissolution of the Nissl substance, which begins, as a rule, in the perinuclear zone and spreads toward the periphery. In fact this process most probably begins much earlier. In the more acute affections, fragmentation seems to take the lead (severe para-

thyroid tetany). In less severe cases, however, (mild tetany, cretinism and functional activity) solution of the granules almost keeps pace with the initial fragmentation. This reaction is indistinguishable under each of the above mentioned conditions. The ultimate expression of this alteration is shown in cells almost devoid of extranuclear chromatin. That which remains is reduced to a very fine state as can be seen from the dust-like consistency of these particles. They possess little or no affinity for the basic stains. In this state of the cell, the contour and outlines become more indistinct.

The nucleus, on the other hand, seems to have maintained its integrity to a greater extent. Other than being slightly swollen and clouded, as described above, it appears quite normal. Complete karyorrhexis and karyolysis was met with in one case only, so little significance can be attached to it. Shifting of the nucleus toward the periphery of the cell occurred no more frequently in the experimental than in the control tissue. Vacuolization of the cytoplasm commonly accompanied the most acute affections, particularly in the Purkinje cells and granule cells of the cerebrum. This was often accompanied by perivascular and pericellular oedema.

From these investigations, chromatolysis has been interpreted as a general reaction of nerve cells; a reaction induced in these elements by conditions opposed to their normal functional equilibrium. If carried beyond certain physiological bounds, this reaction may be looked upon as pathological and extreme conditions result in degeneration and death of the cell. The severity of the opposing factors, and consequently the rapidity with which the changes are produced, do exercise a profound influence, but express themselves quantitatively rather than qualitatively. This point of view was expressed by Van Gehucten[1] some years ago.

[1] Van Gehuchten, *La Cellule*, 1897, xiii.

2856

Rate of absorption of hexoses and pentoses from peritoneal cavity.

CARL F. CORI and HILDA L. GOLTZ.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

It is assumed that the normal mechanism of absorption from the intestine is dependent on the specific function of the epithelial cells. This assumption is based on the observation that a destruction of the epithelial cells by heat or by sodium fluoride changes the mechanism of absorption. The objection to such experiments is the rather crude treatment to which the intestine is subjected.

It has been shown previously[1, 2] that different hexoses and pentoses are absorbed at widely different rates from the intestine. This has been taken as an indication that the intestinal membrane is possessed of a high degree of selective permeability for sugars. It has also been found that if the amount of sugar absorbed is plotted against time a straight line is obtained. If one ascribes these phenomena to the specific function of the epithelial cells of the intestine, then the peritoneal cavity, which has a very different epithelial lining, should show an entirely different mechanism of absorption of sugars.

Experimental.

Full grown mice, not differing by more than 1 to 2 grams in weight, were used. One cc. of 20 per cent solutions of the different sugars, warmed to 38° C., was injected. By using very fine needles, fluid did not escape from the peritoneal cavity after the injection. The mice were killed by decapitation and completely skinned. The carcass was placed in a beaker, the peritoneal cavity cut open and washed out with successive portions of hot, distilled water. The washings were poured into a 200 cc. volumetric flask and after precipitation with colloidal iron, the sugar was determined in an aliquot part of the filtrate. If the mice were killed immediately after the injection from 95 to 97 per cent of the sugar was recovered from the peritoneal cavity.

[1] PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 495, 497.

[2] *J. Biol. Chem.*, in press.

TABLE I.

The rate of absorption of a 20 per cent glucose solution from the peritoneal cavity of mice. 1 cc. or 200 mg. were injected and the animals killed 15, 30 and 45 minutes later.

Length of absorption period.	Amount of glucose absorbed.	Remarks.
minutes.	mg.	
15	74	Average of 20 experiments.
30	101	" " " "
45	108	" " " "

Table I shows that the rate of absorption of sugar from the peritoneal cavity diminishes more and more the longer the absorption is allowed to proceed. This is in marked contrast to the intestine, where the rate of absorption remains entirely constant.

TABLE II.

Comparison of the rate of absorption of hexoses and pentoses from the peritoneal cavity of mice. 1 cc. of 20 per cent sugar solutions or 200 mg. were injected and the animals killed 15 minutes later.

Type of sugar.	Amount of sugar absorbed	Ratio (Glucose=100)	Remarks.
	mg.		
d—Galactose	73	98	Average of 8 exper.
d—Glucose	74	100	Average of 20 exper.
d—Fructose	71	96	Average of 9 exper.
d—Mannose	72	97	Average of 13 exper.
l—Xylose	75	101	Average of 7 exper.
l—Arabinose	76	102	Average of 10 exper.

It is evident from Table II that the different sugars investigated are absorbed at the same rate from the peritoneal cavity. This is again in contrast to the intestine, where, if glucose is taken as 100, galactose gives a ratio of 110, fructose of 43, mannose of 19, xylose of 15 and arabinose of 9.

Conclusions.

1. Sugar is absorbed from the peritoneal cavity at a diminishing rate.

2. The peritoneal cavity is equally permeable for the different sugars investigated.

3. It is suggested that the different mechanism of absorption of sugars from the intestine and the peritoneal cavity is due to the different epithelial lining of these two membranes.

2857

The permeability of liver and muscles for hexoses and pentoses.

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[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

Preceding work has shown that the epithelial cells of the intestine are selectively permeable for different sugars, while the epithelial cells of the peritoneal cavity are equally permeable for all sugars. It seemed of interest to study how other cells of the body, especially those of liver and muscle, behave in this respect. For this purpose sugar was injected intravenously and the animals killed in very short time intervals after the injection. By determining the ratio between the sugar concentration in the blood and in the different tissues conclusions as to the permeability of the tissues could be drawn.

Experimental

Full grown white mice, previously fasted for 24 hours, were used. The injections were made into the tail vein and were timed with a stop watch. Four tenths cc of a 15 per cent sugar solution were injected within 14 to 18 seconds. The animals were then killed exactly 1, 2 and 3 minutes after the start of the injection. Liver and muscles were quickly removed and frozen with compressed CO_2. The organs were weighed in the frozen state and ground up with ice cold 2 per cent HCl. The grinding of the muscles was facilitated by the addition of a small quantity of sand. The proteins were precipitated with 5 per cent H_gCl_2 and the samples were left standing for 1 to 2 hours. After the removal of the mercury in the usual way and neutralisation, the sugar was determined in an aliquot part of the filtrate.

TABLE I.

The permeability of liver and muscles of mice for hexoses and one pentose. Four tenths cc. of a 15 per cent sugar solution, or 60 mg., were injected intravenously and the animals killed 1, 2 and 3 minutes after the start of the injection. Each figure is an average of 2 to 3 experiments.

Type of Sugar	1 minute			2 minutes			3 minutes		
	Blood sugar.	Liver sugar.	Muscle sugar.	Blood sugar.	Liver sugar.	Muscle sugar.	Blood sugar.	Liver sugar.	Muscle sugar.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
d—Galactose	0.980	0.761	0.223	0.861	0.683	0.235	0.759	0.641	0.272
d—Glucose	1.01	0.718	0.243	0.847	0.670	0.240	0.722	0.657	0.279
d—Fructose	1.07	0.756	0.213	0.865	0.681	0.212	0.741	0.631	0.303
d—Mannose	1.02	0.746	0.268	0.850	0.719	0.221	0.737	0.672	0.271
l—Arabinose	1.09	0.715	0.214	0.841	0.669	0.237	0.750	0.622	0.262

TABLE II.

The ratio between sugar concentration in the blood and in liver and muscle has been calculated from the figures in Table I. The blood sugar concentration has been taken as 100.

Type of sugar.	1 minute		2 minutes		3 minutes	
	Liver sugar.	Muscle sugar.	Liver sugar.	Muscle sugar.	Liver sugar.	Muscle sugar.
d—Galactose	77.6	22.8	79.3	27.3	84.5	35.8
d—Glucose	71.1	24.1	79.1	28.3	91.2	38.6
d—Fructose	71.5	19.9	78.7	24.5	85.1	40.8
d—Mannose	73.2	26.2	84.5	26.0	91.1	36.8
l—Arabinose	65.5	19.6	79.5	28.2	83.0	34.9

The data of Tables I and II indicate that the cells of liver and muscle are not selectively permeable for the different sugars investigated. The variations in the different values are within the limit of error of the method.

It is interesting to note how extremely rapidly the sugar leaves the blood stream. A mouse of 20 grams has a blood volume of about 1.5 cc. Since 60 mg. of sugar in 0.4 cc fluid were injected, the blood sugar concentration should rise to about 3 per cent. Yet, within 1 minute from the start of the injection the blood sugar concentration is only 1 per cent. In the following 2 minutes the blood sugar diminishes very slightly, when compared with the initial, abrupt fall. The sugar concentration in the liver also decreases in the second and third minute after the injection. This indicates that an equilibrium between the sugar concentration of the blood and of the liver is reached in 1 minute after the injection. The sugar concentration in the muscles increases slightly in the second and markedly in the third minute after the injection. An equilibrium between the sugar concentration of the blood and of the muscle is not yet reached. It may be concluded that the muscles are decidedly less permeable for sugars than the liver. This may be due to the smaller blood supply of the muscles as compared with that of the liver.

Summary

1. When 60 mg. of sugar are injected intravenously into mice of 20 gram body weight an equilibrium between the sugar concentration of the blood and of the liver is reached in 1 minute after the start of the injection.

2. Different hexoses and the one pentose examined reach this

equilibrium at the same rate and hence penetrate with equal rapidity into the liver cells.

3. The muscles are less permeable for sugar than the liver. The ratio blood sugar: liver sugar : muscle sugar, 3 minutes after the injection, is of the order, 100 : 87 : 37.

4. The different sugars investigated penetrate into the muscle cells at the same rate.

2858

The tolerance of rats for intravenously injected glucose.

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It has been found that rats absorb from the intestine 1.78 grams glucose per kilogram body weight per hour without showing glycosuria. Since, also, equal amounts of sugar were found to pass from the intestine into the blood stream in equal periods of time, the absorption is comparable with a continuous intravenous infusion at a constant rate. Woodyatt, Sansum and Wilder[1] determined the glucose tolerance of normal rabbits, dogs and men by the continuous intravenous infusion method. They found that if glucose was infused at a rate of 0.85 gram per kilogram an hour, no glycosuria was produced, even if the infusion was extended over many hours. This is obviously a much lower tolerance than that observed on rats during intestinal absorption. There seemed to be two explanations possible. Either the rat has a higher glucose tolerance than rabbit, dog or man, or, the glucose tolerance, when tested by the intestinal route, is higher than when tested by the intravenous route. In order to see which of the two explanations is correct the sugar tolerance of rats was tested by the intravenous infusion method.

Experimental.

Non-fasting male and female rats were used. The experi-

[1] Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am. Med. Assn.*, 1915, lxv, 2067.

ments could not be made without general anesthesia. Fortunately, amytal (iso-amy-ethyl barbituric acid) is a narcotic which is known to have no effect on carbohydrate metabolism, an observation which we have verified on previous occasions. A 2 per cent solution of amytal was made. Seven and five-tenths to 8 mg. per 100 gram body weight, injected intraperitoneally, produced a very satisfactory narcosis.

The infusions were made into the femoral vein. The device used to secure a constant, very slow flow of the sugar solution from the burette was that described by Burn and Dale.[2] A 10 cc. burette, graduated in 0.02 cc., was used and readings were taken every 2 minutes. The rate of infusion could be kept constant within 0.02 cc. as is shown by the following figures. Rat No. 6 of Table I was infused with an 8.2 per cent glucose solution, 4.08 cc. being delivered from the burette in 1 hour. The burette readings in cc. in 2 minute intervals, were as follows: 0.13, 0.13, 0.14, 0.13, 0.13, 0.14, 0.13, 0.13, 0.13, 0.13, 0.14, 0.13, 0.14, 0.14, 0.15, 0.14, 0.13, 0.14, 0.14, 0.14, 0.15, 0.15, 0.14, 0.14, 0.13, 0.13, 0.13, 0.14, 0.13, 0.13.

After the infusion had been kept up for 1 hour a small opening was made in the abdominal wall and the bladder pressed out gently. At the same time 0.2 cc. of blood were collected from the other femoral vein. In most instances the infusion was extended for 1 or 2 more hours.

The urine was treated with Lloyd's reagent in order to remove interfering substances and the sugar was determined by the Hagedorn and Jensen method. Rats fasted for 48 hours excrete a urine which contains 0.35 mg. reducing substances per 100 grams body weight per hour. It is doubtful whether these reducing substances are actually sugar. During the absorption of glucose from the intestine, rats excrete 1.36 mg. reducing substances per 100 gram body weight per hour and have a blood sugar ranging from 0.170 to 0.203. An excretion of more than 2 mg. reducing substances per 100 gram body weight per hour, which coincides with a blood sugar concentration above 0.200, was taken as indicating glycosuria.

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[2] Burn, J. H., and Dale, H. H., *J. Physiol.*, 1924, lix, 164.

TABLE 1.

The intravenous tolerance of rats during amytal narcosis. An excretion of more than 2 mg. of sugar per 100 gm. body weight per 1 hour indicates glycosuria.

No.	Weight and sex	Length of infusion period	Percentage of glucose solution	CC. infused	Glucose per 100 gm. body weight per 1 hour	Blood sugar	Sugar excreted per 100 gm. per 1 hour	Rate of infusion below or above tolerance
	gm.	Min			gm.	mg.	mg.	
1	F. 132.5	90	8.00	2.02	0.081	—	0.37	below
2	M. 131.4	60	7.55	1.52	0.087	—	0.57	below
		60	7.55	2.05	0.118	143	0.77	below
3	M. 146.8	60	9.35	2.15	0.137	167	0.94	below
4	M. 126.9	54	9.25	3.09	0.250	215	2.37	just above
5	F. 128.5	60	9.25	4.88	0.351	337	20.85	above
		60	Infusion was stopped			151	5.56	—
6	F. 126.6	60	8.20	4.08	0.264	254	8.87	above
		54	8.20	3.90	0.252	315	32.60	above
7	M. 152.7	60	9.30	3.87	0.235	201	1.95	below
		60	9.30	3.90	0.237	209	1.94	below
8	M. 136.4	80	9.00	4.44	0.219	—	0.99	below
9	F. 141.9	60	14.85	1.97	0.206	152	1.00	below
		60	14.85	2.61	0.272	—	14.30	above
		60	14.85	2.46	0.257	376	31.14	above
10	F. 146.2	60	15.05	2.16	0.222	—	8.88	above*
		60	15.05	2.19	0.225	266	13.95	above*
11	M. 155.2	60	15.20	1.82	0.178	147	0.77	below
12	M. 153.6	60	15.20	2.24	0.221	—	1.55	below
		60	15.20	2.12	0.210	—	1.22	below
		60	15.20	2.27	0.224	151	1.10	below

*Vaginal smears showed that this rat was at the height of oestrus.

It will be seen from Table 1 that the intravenous tolerance of rats is between 2.2 and 2.5 gram glucose per kilogram body weight per hour. This is a tolerance of about two and one half times as high as that found by Woodyatt on rabbits, dogs and men. Rat No. 10 falls out of the series. This animal was at the height of oestrous, as was revealed by vaginal smears. Whether this is a mere coincidence or whether the glucose tolerance is lowered during oestrous can only be decided after a larger number of animals has been examined.

The intravenous tolerance is a measure of the maximum rate at which glucose can be metabolized in the body. It is an interesting physiological fact that the maximum rate at which glucose can be absorbed from the intestine is markedly below the intra-

venous tolerance rate. Rats, fasted for 48 hours, absorb glucose at a rate close to 1.78 gram per kilogram per hour or 1.54 gram per kilogram per hour if based on the body weight before fasting.

Summary.

The intravenous tolerance of non-fasting rats during amytal narcosis is between 2.2 to 2.5 gram glucose per kilogram body weight per hour.

2859

Significance of change in oxygen absorption after insulin in normal rabbits.

ESTELLE E. HAWLEY and JOHN R. MURLIN.

[*From the Department of Vital Economics, University of Rochester, Rochester, N. Y.*]

Respiration experiments by the closed circuit method employing an apparatus which gave perfect control checks are reported upon twenty fasting rabbits. Seventeen of these received an injection of insulin subcutaneously which reduced the blood sugar an average of 62 mg. in two hours. One of two basal periods of 45 to 60 minutes each were obtained before giving insulin, and two periods of at least 45 minutes each, in all cases, and four periods in five of the cases, were obtained after insulin. There was no rise but often a fall in respiratory quotient during the first period up to one hour after insulin. In the second period, however, the average respiratory quotient was 0.98 in contrast with the average of 0.74 in the pre-insulin period. In the third period after insulin, the respiratory quotient returns to the normal level and persists also in the fourth period. The oxygen absorption on the average rises the first hour and falls considerably below the pre-insulin level the second hour. The CO_2 elimination rises the first hour and the average for all the animals rises still farther the second hour. But the average for the five animals studied longest the CO_2 does not rise farteher in the second period, and falls

toward but does not quite reach the pre-insulin level in the third and fourth periods.

Calculations of the metabolism, by the Zuntz and Schumburg method, of several animals in which the urinary nitrogen was known, shows in the first hour after insulin an average increase amounting to 16 per cent. In the second period terminating at 1¾ hours after insulin, there is an abrupt change in the metabolism from fat to carbohydrate. For example, in the average for five rabbits the change is from no carbohydrate to 1.36 grams per hour and from 0.8 grams fat to 0.06 grams per hour. These changes are believed to be the characteristic action of insulin whether in normal or diabetic animals. The diminished oxygen absorption for the second period is not due to depression but to the fact that additional oxygen is made available for combustion by bringing carbohydrate into the metabolism in place of fat. The diminished absorption in several instances exactly balances the additional oxygen thus made available. The amount of sugar burned under the influence of insulin is far greater than can be accounted for by the most liberal estimate, by the disappearance from the blood, or, from the blood, lymph and tissue fluid combined. Hence the disappearance of glycogen from the liver and other organs of normal animals, as reported by many authors.

2860

Osseous and muscular changes in thyroidectomized sheep.

S. A. GOLDBERG and S. SIMPSON.

[*From the Laboratories of Comparative Pathology and Experimental Physiology, Cornell University, Ithaca, N. Y.*]

In about thirty cretinized sheep and goats it was noted that the abdomen and the digestive tract were distended. Several showed either abdominal hernia or prolapse of the rectum. A case of cretinism in a human subject three years of age observed by one of us also had distension of the abdomen and prolapsed rectum. The skeleton in all of them was smaller, in some instances being less than half the size of the controls.

In the femurs of three cretinized sheep and one goat, the marrow of the shaft was red and the epiphyseal cartilages intact and irregular. The bones were fragile. Histologically, there was an increase in hemopoietic marrow with large numbers of megakaryocytes, formative erythroblasts and granular leucocytes. The center of the epiphyseal cartilage showed absence of nuclei, the presence of a homogeneous blue area, while at the periphery there were proliferating cartilage cells. In the control of the same age the epiphyseal cartilages had entirely disappeared.

Chemical analysis of one of these, performed by R. C. Miller, showed the per cent of ash in dry matter 32.564, while in the control 37.065.

The skeletal and cardiac muscle was constantly pale and flabby as compared with the controls. Histologically there was practically complete absence of the cross striations with the nuclei in the sarcolemma smaller and more numerous than in the controls. The cardiac muscle showed an increased number of what was thought to be swollen Purkinje cells. They contain large pale staining nuclei with much cytoplasm having longitudinal striations at the periphery. It is the opinion of Dr. B. F. Kingsbury that they are embryonal myocardial fibres. It now seems possible that they are premature muscle fibres that have degenerated.

The plain muscle of the aorta showed areas of marked atrophy with vacuolation of the remaining fibres. It seems possible that

this change in the muscle fibres precedes calcification previously described in these cretins[1].

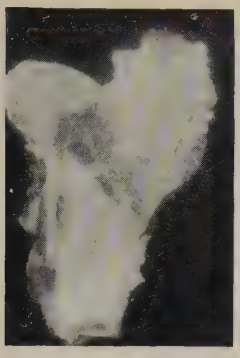

Fig. 1.
Head of femur, Sheep I, Control.

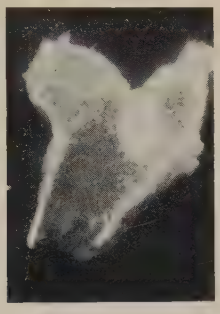

Fig. 2.
Head of femur, Sheep I, Cretin. Note the dark marrow of the shaft and the epiphyseal cartilage which is absent in the control.

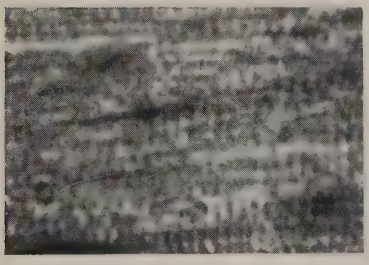

Fig. 3.
Skeletal muscle, Sheep I, Control. Showing normal cross striations.

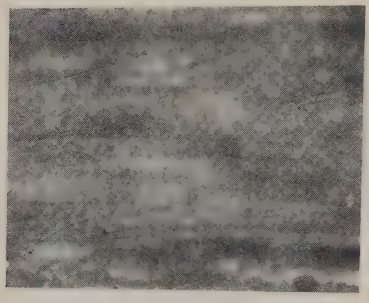

Fig. 4.
Skeletal muscle, Sheep C, Cretin, of the same age as Fig. 3. Showing absence of the cross striations.

These changes seem to indicate that as a result of the thyroidectomy there are degenerative changes following an arrested development of the osseous and muscular tissues.

[1] Proc. Soc. Exp. Biol. and Med., 1924, xxi, 567.

Missouri Branch

St. Louis University School of Medicine October 28, 1925.

2861

Fluid crystals and meristematic growth.

FRANCIS O. SCHMITT and WILLIAM H. CHAMBERS
(Introduced by M. T. Burrows).

[*From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University School of Medicine, St. Louis, Mo.*]

In an attack upon certain phases of the problem of meristematic growth, a cytological investigation of the cells of the squash root tip was made. Tissues fixed in fluids which preserve lipoidal structures, such as formalin-bichromate mixture, and osmic acid revealed structures which warrant description.

The growing tips were fixed by two to three weeks impregnation in 2 per cent osmic acid, after the Kopsch-Mann technique. The sections were mounted in balsam unstained. In such preparations there appeared granules of varying sizes but of uniformly high refringency. These granules were practically round, and in ordinary light the centers appear lighter than the periphery. They are present in all parts of the tip. In the growing point they are small and occur from three to six to the cell and are usually clumped in one corner or are arranged along the cell wall. Some cells, however, appear to be devoid of these characteristic granules. In the highly vacuolated cells they are much larger and fewer to the cell than in the tip. In such cases they are almost invariably found to lie at the periphery of the vacuole, close to the cell wall. The object in making these osmic acid preparations was to determine whether any structures are present which might correspond to the Golgi bodies of animal cells. It is not certain whether these granules are actually Golgi bodies. Other granules which are not birefringent are also present and the probability is that they represent different stages in the metabolic activity of

the cell. There is no evidence of a canalicular apparatus such as Bensley[1] found in the cells of the onion root tip.

When these granules were studied with the polarizing microscope they were found to be uniaxial sphaero-crystals. A very good imitation of these crystals may be obtained by making a thin smear of lecithin on a slide and treating the smear with 2 per cent osmic acid for a short time. Upon examination of such a preparation in polarized light, each of the many highly refringent droplets displays a black cross in the center, if the axis of the crystal is parallel to the optical axis of the microscope.

These birefringent droplets belong to the class of substances first termed by Lehmann, fluid crystals. Since then, Friedel[2] has suggested that this state of matter be called the mesomorphic state, being neither fluid nor crystalline, but possessing many of the properties of both states. The birefringent structures found in these cells seem furthermore to be in the state corresponding most closely to the subdivision called by him the nematic state.

The significance of the fact that many cell structures such as lipoidal granules, mitochondria, and perhaps the Golgi bodies are in the mesomorphic state in normal cell function is just beginning to be appreciated by cytologists and cell physiologists. In a valuable review of the subject, Giroud[3] has very recently called attention to the formative and proliferative power of substances in this state, with especial emphasis on mitochondria. In view of the rapid rate of division and growth of the meristematic cells, the suggestion is offered that the fluid crystalline bodies found in these cells may be important factors in this high rate of activity.

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1 Bensley, R. R., *Trans. Chicago Path. Soc.*, 1910, viii, 78.

2 Friedel, G., *Annales de Physik*, 1922, xviii, 273.

3 Giroud, A., *Archiv. d'Anat. Mic.*, 1925, xxi, 145.

2862

A clinical experiment in subacute streptococcus endocarditis.

R. A. KINSELLA and O. GARCIA.

[*From the Department of Internal Medicine, St. Louis University School of Medicine, St. Louis, Mo.*]

Permission having been obtained, an attempt was made to study the effect in a patient with Subacute Streptococcus Endocarditis of inoculation with autogenous, living organisms. Without previous observations, and without knowing whether the effects would harm or benefit the patient the utmost caution was used with regard to dosage. Four inoculations were made within a period of four weeks. The first two injections were intracutaneous, the second subcutaneous and the fourth consisted of infected agar injected subcutaneously. The first injection consisted of about two cubic millimeters of broth culture attenuated by freezing and melting five times so that the resulting culture yielded weakly growing subcultures. No reaction followed this injection up to six days.

The second injection consisted of four cubic millimeters of broth culture that had remained in the ice box four days. No reaction followed during the same interval.

The third injection consisted of five cubic millimeters, injected subcutaneously, of twenty-four hour broth culture. No reaction followed.

Ten days later 1 cc. of melted agar freely inoculated with twenty-four hour broth culture was injected subcutaneously. No reaction followed.

A moderate clinical improvement could not certainly be ascribed to this procedure and all inoculations were discontinued one month before death.

Following these clinical observations, rabbits were inoculated with cultures intradermally and with infected agar subcutaneously, using the same streptococcus. No reaction followed immediately or at any time up to ten days after inoculation.

Although it is easy to reproduce the disease in dogs by using a non-hemolytic streptococcus obtained from a case of Acute Rheumatic Fever, attempts were unsuccessful in three dogs when strains from two cases of Subacute Streptococcus Endocarditis were used.

It is further interesting to note that in the experiments in dogs in which the more virulent streptococcus was employed, the animals suffered a rapidly rising bacteriemia while patients displayed a constant number of colonies in culture. But when such animals, two in number, were infected with the same streptococcus previously agglutinated, the bacteriemia was more constant like that of patients.

Strains from Acute Rheumatic Fever, when injected subcutaneously in rabbits produce abscesses, and agar infected with such a strain and injected subcutaneously in dogs produces a similar result.

It seems therefore, that cultures from patients with Subacute Bacterial Endocarditis, are in effect "sensitized" or agglutinated cultures.

2863

Some metabolic aspects of calcium therapy.

A. P. BRIGGS.

[*From the Department of Medicine, St. Louis University School of Medicine.*]

Excretion of the various mineral elements was followed for a period of ten days after a diet of known inorganic composition. During the last four days of the experiment a measured amount of calcium acetate was taken by mouth.

Balance sheets, for the four day period of calcium acetate and the four day period preceding, show that calcium has no effect on the metabolism of any element except phosphorus. There was no increase in the excretion of sodium as after the administration of potassium salts. The amount of phosphorus excreted in the urine was decreased and the amount excreted in the feces was increased during the calcium acetate period. With this decrease in the phosphoric acid load on the kidney the amount of ammonia excreted in the urine was also decreased.

In another experiment similarly conducted, it was found that the administration of potassium or magnesium acetates had no influence on the partititon of phosphorus in the urine and feces, whereas the effect of calcium chloride was similar to that of calcium acetate.

2864

"Areas of lowered acuity" in relation to quantitative tests on bone and air-transmitted sound.

A. G. POHLMAN and F. W. KRANZ.

[*From the St. Louis University, St. Louis, Missouri, and the Riverbank Laboratories, Geneva, Illinois.*]

Adult individuals often show an area of decreased acuity for air transmitted sounds in the range between 2400 and 3000 pps. in the auditory field of frequency. Such ranges of decreased acuity are often sharply defined and may require a stimulus up to 500 or 1000 times the normal minimum to elicit response. This finding might be explained by almost any of the numerous theories of end organ behavior on the basis of a local defect in the basilar or tectorial membranes.

The first subject of the series of the present investigation showed a bilateral defect in this frequency range which is known to have been constant for the past four years. When tested with the bone activating telephone receiver, this individual showed a decreased bone acuity through the same range of frequency. The second subject has had a unilateral defect in air acuity for at least five years, and on testing with the bone activating telephone it was found that he also showed a refect in bone acuity. The third subject displayed no lowered air acuity through this range but did show a decreased bone acuity similar to the two previous cases. Four more subjects with normal air acuity did not show the bone acuity defect. In order to eliminate the possibility of any influence of the particular bone activating receiver on the results, the tests were repeated with another instrument of different construction and the above results were verified. The same results were obtained on repetition of the tests. All bone acuity tests were made from the forehead.

The findings will be discussed in detail in the Laryngoscope.

The report is of peculiar significance because the defect which might have been interpreted in terms of internal ear reaction if occurring in both bone and air acuity, cannot be so interpreted because it can occur in the bone acuity without being present in

the air acuity. We can offer no explanation for it, but the demonstration of this effect in the sound transmission apparatus is a serious obstacle to the differential diagnosis between a conduction and a perception deafness.

Pacific Coast Branch

University of California

October 24, 1925

2865

The presence of bacterial microorganisms within human gingival tissue in gingivitis.

T. D. BECKWITH.

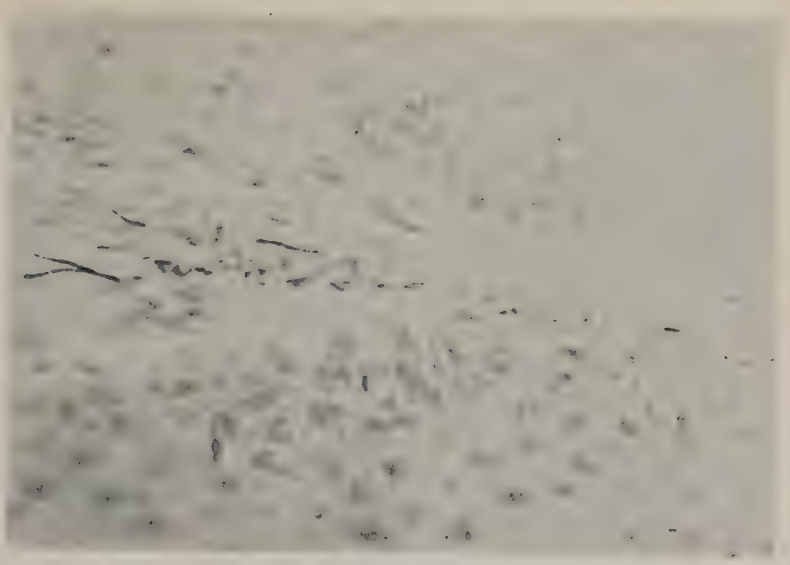
[*From the Department of Bacteriology, University of California, and the California Stomatological Research Group.*]

Human gingival tissue has been obtained at biopsy from subjects showing gingivitis. This has been fixed and sectioned in paraffin. Various methods suitable for pathological histological examination and for the demonstration of microorganisms have been followed.

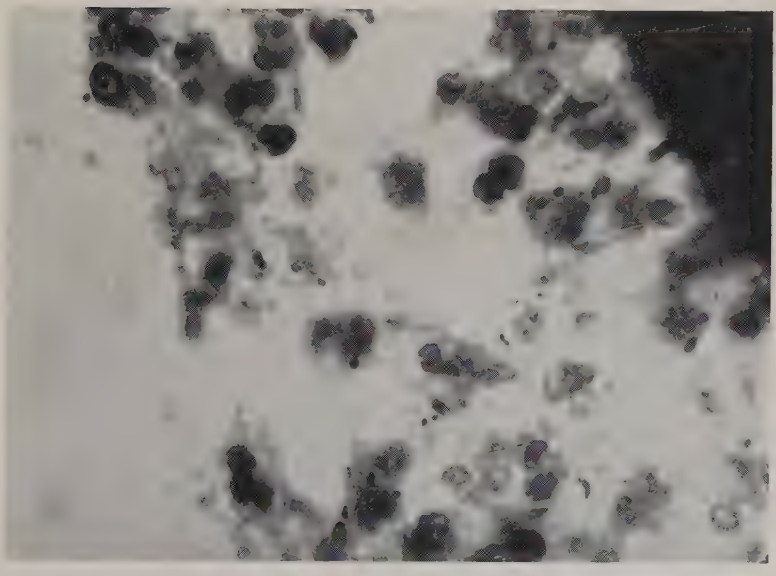
Such tissues show a subacute inflammatory process characterized by the presence of large numbers of lymphocytes and plasma cells. Likewise, there are present within this tissue and closely adjacent to lymph channels, a variety of bacterial forms, especially streptococci and what appear to be diptheroids. The accompanying photomicrographs indicate some of these.

Although it has been denied by certain authors that there is any phagocytosis of microorganisms by cellular elements within this region, it will be noted that there is definite evidence of such reaction in one of the photographs submitted. Careful microscopic examination does not convince us that the engorging cell is the polymorphonuclear leucocyte. Possibly the macrophage may be implicated.

The forms thus demonstrated within gingival tissue also are living and in part at least may be cultivated as follows: The gum surface is first sterilized by careful application of a solution consisting of 2 per cent crystal violet plus 2 per cent brilliant green



Coccus and rod forms within human gum tissue in gingivitis.



Phagocytosis of coccus forms within human gum tissue in gingivitis.

in 50 per cent alcohol. Physiological saline is then injected and then aspirated by means of a hypodermic syringe with a 27 guage needle.

The fluid thus obtained is then cultured in deep beef infusion glucose blood broth and in brain medium. It is then streaked out on blood agar plates.

To date, 149 aspirations have been made from gingival tissue of twenty-four men at San Quentin Penitentiary. These 149 tests have yielded positive cultures in ninety-seven instances or 65 per cent. The distribution of these cultures according to morphology and cultural reactions together with Holman's classification for the streptococci is as follows: Staph. albus, 53; Staph. sureus, 1; Strep.pyogenes, 13; Strep. equimus, 3; Strep. angiosus, 15; Strep. salivarius, 28; Strep. mitis, 26; Strep. infrequens, 3; Strep. ignavus, 6; Strep. non-haemolyticus II, 1; Strep. equi, 1; Micrococcus gasogenes, 1.

Dr. G. W. Simonton, Miss Adrienne Williams, and Miss E. J. Rose have been associated with me in this work and in more complete publication will be given coauthorship in the subdivisions.

2866

The effect of dry grinding upon gels.

CARL L. ALSBERG and ELIZABETH P. GRIFFING.

[*From the Food Research Institute, Stanford University, California.*]

The writers have shown that dry grinding in a pebble mill renders starch largely soluble in cold water[1]. They are now able to report that similar treatment renders gelatin temporarily soluble in cold water. Solutions thus prepared set to a gel after a time. The water insoluble colloids, gliadin and glutenin, which together form wheat gluten, are not rendered materially soluble by sixteen hours grinding of the flour from which they are prepared, though their physical properties are changed. If gluten be washed from

[1] PROC. EXP. BIOL. AND MED., 1924, xxii, 60.

such flour in the usual way, and if its swelling in acid be studied by the method of Upson and Calvin[2], it is found that the gluten, prepared from the excessively ground flour, swells less than the control gluten prepared from the same flour before it was subjected to overgrinding. The difference is so definite that it is possible by mere inspection to distinguish the two kinds of swollen gluten. The control gluten, as it swells in weak acid, becomes translucent, slimy and runny; while gluten from the same flour after grinding, remains more opaque, swells less and is much firmer to the touch.

It is thus apparent that rather mild mechanical treatment is capable of affecting profoundly the physical properties of the biologically important gel-forming colloids so far studied. It is difficult to harmonize the results herein recorded with the hypothesis defended by Katz[3] that swelling is merely a special case of solution. They rather speak for the view that swelling is dependent—at least in some degree—upon structure and that grinding possibly modifies this structure. The character of the structure thus modified may be relatively coarse, such as a reticulum represented by the continuous phase. Or it may be a colloidal or even a molecular structure that is broken. Or, finally, there may be jumbling of an orderly arrangement of the molecules such as the X-ray examinations of Herzog, of Katz[4] and of Sponsler[5] have made probable in certain gels. Indeed Sponsler[5] has reported that the X-ray spectrum of starch is destroyed by grinding. Investigations are in progress to determine which of these possibilities is the most probable.

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[2] Upson, F. W., and Calvin, J. W., *J. Am. Chem. Soc.*, 1925, xxxvii, 1295.

[3] Katz, J. R., *Ergeb. exakt. Naturwissenschaften*, 1924, iii, 316.

[4] Katz, J. R., op. cit.

[5] Sponsler, Clenus Lee, *Am. J. Bot.*, 1922, ix, 471.

2867

Diameter measurements of urinary casts.

C. W. BARNETT and G. D. BARNETT.

[*From the Department of Medicine, Stanford Medical School, San Francisco, California.*]

Diameters of urinary casts in a number of pathological sediments have been measured and their frequency distributions studied. In most of the sediments measured the diameters range from about 8 microns to 40 or 50 microns, with a mean value of from 19 to 24 microns. Frequency polygons obtained from measurements of 100 to 300 casts grouped in class intervals of 3 microns are usually unimodal and slightly skew, with the long tail in the direction of the greater diameters. This asymmetry is probably due to the fact that very small casts are difficult of

Mean Value and Dispersion of Cast Diameters.

Case	Type of Renal Disease	Mean Diameter.	Standard Deviation
1	Hemorrhagic, initial	16 microns	4
2	Hemorrhagic, latent	19	7
3	Hemorrhagic, active	19	5
		20	6
4	Hemorrhagic, active	19	7
5	(Cardiac decompensation)	21	7
		20	6
6	Hemorrhagic, active	21	6
7	Hemorrhagic, latent	20	6
		20	6
		21	6
8	Hemorrhagic, latent	20	7
9	Hemorrhagic, active	20	5
		21	7
		23	6
10	Hemorrhagic, active	19	4
11	Cryptic, degenerative	22	7
12	Hemorrhagic, active	19	4
		23	6
13	Hemorrhagic, latent	24	7
14	Hemorrhagic, terminal	25	7
		28	9
15	Degenerative (jaundice)	31	15
		30	12
16	Hemorrhagic, terminal	35	13
17	Hemorrhagic, terminal	41	13
		42	14
18	Hemorrhagic, terminal	50	18

identification. A few of the sediments contained high percentages of broad "renal failure" casts described by Addis[1]. Plots from these sediments show high mean diameters and high degrees of dispersion, but are not otherwise significantly different from the other curves. In general the dispersion increases with increasing mean diameter. The broadest casts seen measured 98 microns.

2868

Hereditary doubling suggesting anomalous chromatin distribution in the mouse.

C. H. DANFORTH.

[*From the Department of Anatomy, Stanford University, California.*]

Mice with six legs appeared about two years ago in a stock which had descended from five individuals and had been inbred for several generations. Since this stock had been subjected to no special treatment, the possibility of the anomaly having a purely hereditary basis suggested itself, and in the fall experiments were undertaken to determine if such were the case.

In the course of the tests many anomalous individuals have been produced ranging from Y-shaped specimens with four hind legs and two tails, to those with a relatively slight degree of doubling in the external genitalia. These may all be referred to as "doubles." Apparently connected with the same manifestation is the appearance of hemorrhagic testes, *spina bifida,* and occasional other anomalies such as microphthalmia. The anomalous individuals are for the most part incapacitated for breeding, so their parents and sibs have been isolated from the rest of the stock and used for the experiments.

At first anomalous individuals were produced about equally by the selected (D) and unrelated (B) strains, but gradually the former began to show an increased incidence which has risen to about 12 per cent (113:805) for the whole A strain and about

[1] Addis, Renal Failure Casts, *J. Am. Med. Assn.*, 1925, lxxxiv, 1013.

20 per cent (45:178) for the best producers. In the meantime doubling has been practically eliminated from the B strain, only two anomalous individuals having appeared among the last 1100 or more young. In a few instances the character of the anomaly has been such as to permit of the production of young, and one mating between two anomalous individuals has been successful. Results of different types of matings are as follows:

Mating	Normal Young	Double Young	
1. Double x Double	13	1	8 %
2. Double x Normal (D strain)	127	10	8 %
3. Normal (D) x Normal (D)	805	113	20%
4. Normal (D) x Normal (B)	348	7	2 %
5. Normal (B) x Normal (B) (more than)	1100	2	2 %

In view of the fact that the animals are all closely related, have been kept in the same room, and received similar treatment, there can be little doubt that the anomaly has a germinal basis which, through selection, has been mostly segregated in the D strain and practically eliminated from the B strain. But the results of the breeding tests do not accord with expectations based on the assumption that the anomaly is conditioned by either one or two dominant, recessive, or cumulative genes. On any theory "non-appearance"[1] must be taken into account as an important element in the situation. A single dominant gene with a somatic "non-appearance" of about 70 per cent would account for all the classes but would not give proportions very close to those observed—20 per cent, 18 per cent, and 15 per cent, instead of 8 per cent, 8 per cent, and 11 per cent in groups 1, 2 and 3. Differential prenatal death rate is also to be considered but is not of itself adequate to explain relative difference between classes. A differential factor favoring the production and function of normal gametes in heterozygous individuals would affect different classes differently and bring the expected numbers closer to those actually observed.

This leads to the tentative hypothesis that we are dealing in this case with some form of chromosomal aberration involving a certain amount of reduplication in chromatin material (a whole chromosome, or part of one) without necessarily any qualitative change in the chromatin material itself. The character of the

[1] *Genetics*, 1924, ix, 206.

trait itself, which is not a very specific entity but involves essentially increases or aberrations in structures which are normally present, might be regarded as rather favoring this explanation. Before this hypothesis, which would bring these cases of mammalian teratology into line with many roughly similar phenomena in the field of botany, can be regarded as more than a suggestion, a great deal of critical data must be accumulated. If it should be substantiated one of the main causes of the observed aberrant ratios would be the frequent failure of cells which receive the extra chromatin element to develop into functioning gametes.

2869

Microelectrodes and micromagnets.[1]

C. V. TAYLOR.

[*From Stanford University, California.*]

Two kinds of microelectrodes and one micromagnet have been perfected and used with very good results.[2] First, it was found that a platinum wire (No. 35, C.P.) inserted into a close-fitting quartz capillary can be drawn over a minute oxy-acetylene flame to a perfectly insulated needle-point less than one micron in diameter. Indeed, the platinum core in this exceedingly fine point may closely approximate the limits of microscopic vision.

The platinum wire at the opposite (undrawn) end extends a few mm. beyond the quartz capillary in order that the former may be annealed to an insulated copper wire of about the same diameter as that of the platinum wire and 2 feet in length. The drawing of the electrodes (see Fig. A, 1 and 2) and the annealing is readily done over the oxy-acetylene microburner (m.b.). About 3 mm. of the needle-tip of the electrode is bent over the flame, at right angles (Fig. A, 3) and the opposite end is sealed with deKhotinsky cement into a glass shank 5 mm. in

[1] Funds for these investigations were generously provided by President Campbell and the Research Board of the University of California.

[2] Taylor, Cataphoresis of Ultramicroscopic Particles in Protoplasm, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 533.

diameter and 35 mm. long (Fig. A, 3, sh). This shank fits into the instrument-holder of a recently designed micro-manipulator.[3] By means of the latter the electrodes are operated with finest precision in a moist chamber on the stage of the microscope.

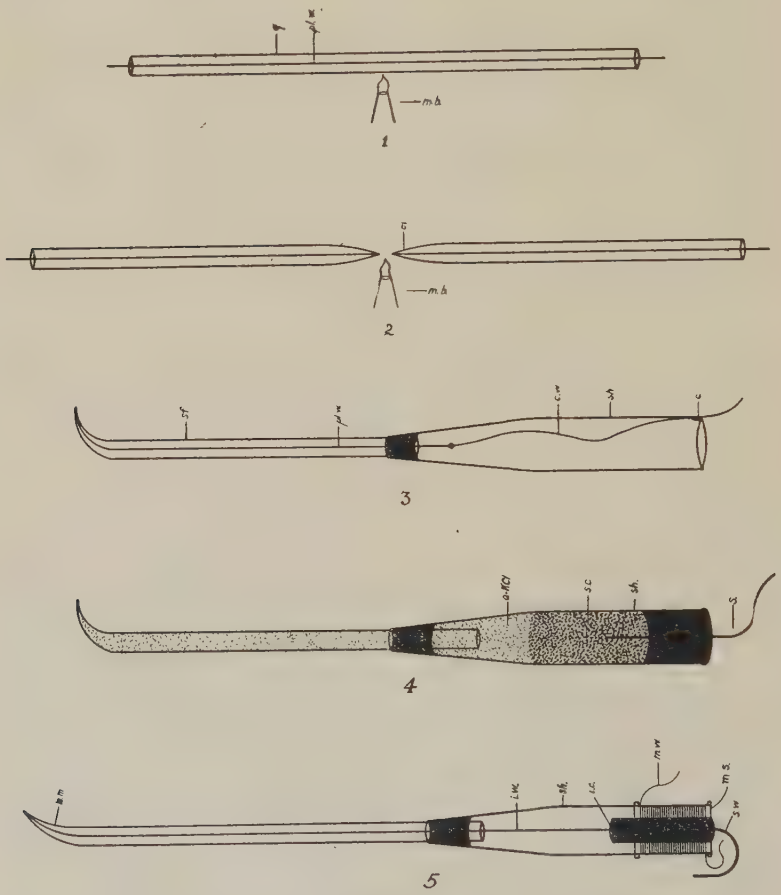

FIGURE A.

Showing construction of Micro-electrodes and Micro-magnets.

a-KCl, 0.5 per cent solution of potassium chloride in dialyzed agar; *c.*, cement; *c.w.*, copper wire; *el.*, electrode; *i.c.*, iron core; *i.w.*, iron wire; *m.b.*, micro-burner; *m.m.*, micro-magnet; *m.s.*, magnet spool; *m.w.*, magnet wire; *pl.w.*, platinum wire; *q.*, quartz capillary; *s.c.*, silver chloride; *s.*, silver wire; *sh.*, shank; *sf.*, shaft; *s.w.*, "shorting" wire.

[3] Taylor, Improved Micromanipulation Apparatus, *Univ. Calif. Pub. in Zool.*, 1925, xxvi, 443.

The second type of microelectrode is non-polarizable. For many purposes the platinum electrode would appear to be entirely satisfactory, but certain experiments make necessary the use of this second type. Peterfi[4] has recently described a microelectrode very similar to this one which has been in use for several months, so that his and mine were designed quite independently. Our methods of constructing these non-polarizable microelectrodes are somewhat different, hence I offer the following procedure:

A micropipette, made preferably from a quartz capillary of about 1 mm. in outside diameter and 50 cm. long, is filled by means of a good Luer syringe with a melted 0.5 per cent KCl solution of carefully dialyzed agar. When the agar solution has thoroughly hardened, the micropipette is sealed with White's dental cement (of low melting point) into a glass shank (Fig. A, sh.) similar to that described above for platinum electrodes. The inner end of the pipette, which should extend only a few mm. into the "bowl" of the shank, is now well covered with a few drops of the melted agar solution. When the agar has thoroughly cooled, the bowl of the shank is almost filled with slightly moist, well pulverized AgCl (C.P.). Into the silver chloride is inserted a No. 20 silver wire of convenient length (about 1 ft.). The end of the "bowl" is then filled and completely sealed with deKhotinsky cement which thereby firmly holds in place the silver wire. It is advisable to insulate the exposed portion of the silver wire by coating it with the deKhotinsky cement or by means of glass beads which can be quickly made by cutting a capillary tube into lengths of 1 cm. each.

After mounting the two electrodes of this type in the micromanipulator they are "shorted" for some time previous to use, in order to obviate any difference of potential.

The E. M. F. is provided by a dry cell of about 1.5 volts (the voltage being precisely determined by means of a Weston Standard Cell). Included in the circuit are two resistance boxes totalling 12,000 ohms and one of 40 ohms, a nitrogen-mercury key and a specially designed Leeds and Northrup galvanometer having a sensitivity of 35,714 megohms.

The micromagnets, consisting of quartz with a soft iron core, are drawn in the same manner and quite as fine as the platinum

[4] Peterfi, *Pflüger's Archiv. Physiol.*, 1925, ccviii, 454.

electrodes described above. Into a close-fitting quartz capillary 30 cm. long is inserted a No. 30 soft iron wire with free ends extending 20 cm. beyond either end of the capillary tube. The magnets are then drawn over the microburner as illustrated in Fig. A. The very tip of the iron core is completely enclosed by the quartz. After slightly curving the drawn ends (Fig. A, 5) each magnet is thereupon mounted and temporarily cemented into the glass shank such that the free end of the iron wire projects 3 or 4 cm. beyond the base of the shank. This permits the soldering of the wire onto one end of the soft iron core (*i. e.,* Fig. A., 5) of the magnet spool (m.s.). This spool, made of wood, is 2.5 cm. long and 1.5 cm. in diameter—a size sufficient to accommodate the winding on of 1500 ft. of No. 36 triple insulated copper magnet wire (m.w.) One end of the soft iron core (4 mm. in diameter) should extend about 3 mm. beyond the spool in order to provide support for the glass shank. Having soldered the iron wire to the projecting core, the glass shank, now unsealed from the micromagnet, is slid over the free end of the iron core whereon it is firmly cemented by means of the deKhotinsky wax. In turn, the micromagnet is permanently cemented in the shank (Fig. A, 5).

The micromagnets, like the microelectrodes, are supported and operated in the moist chamber by means of the micromanipulator.

It is evident that these electrodes and magnets afford a means for the study of the electric and magnetic properties of protoplasm in the interior of a living cell, thus eliminating an unknown factor—the cell-membrane. But they also provide means for an independent study of this unknown factor. It is believed that other uses also may be made of these microinstruments.

2870

The blood fibrin in canine anaphylaxis.

EDWIN W. SCHULTZ and GLADYS NEWNAN.

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.*]

Attention[1] was called to the fact that the delay or loss of the coagulability of the blood in canine anaphylaxis is apparently due to a reduction in the number of blood platelets, resulting in a reduction in the amount of thromboplastin necessary for the formation of a coagulum. With this in mind it was found possible to make a quantitative study of the blood fibrin by means of the simplified technique of Foster and Whipple[2] plus the addition of a thromboplastic agent to the clotting solution. This was supplied either in the form of a suspension of platelets, prepared as indicated in the previous paper,[1] or of a commercially prepared thromboplastin (Squibbs). Since the latter was the most convenient, this was generally used. The commercial product was filtered through a Gooch filter and was added in uniform quantities, 1 or 2 cc., to the clotting solution into which the oxalated plasma had been discharged. All the plasma samples, including the normal, from a given animal were treated in the same manner.

The animals were sensitized to horse serum, 0.3 cc. per kilo subcutaneously, and the shocking dose, 1 or 2 cc. per kilo of animal weight, was administered by intracardial injection. Anaphylaxis in dogs so injected almost invariably terminates fatally and marked pulmonary fixation, generally considered uncommon in canine anaphylaxis, is frequently observed at autopsy together with the hepatic congestion. The blood samples were also obtained by cardiac puncture.

Our results show that as a rule there is a well marked decline in the fibrin values immediately after the drop in blood pressure. The initial abrupt decline is generally followed by a more gradual one of variable duration. In animals which live sufficiently long, a gradual shift towards the normal level generally becomes evident. It is noteworthy that corresponding to the initial drop in

[1] Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 343.

[2] Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lvii, 365.

Fibrin and Hematocrit Values during Shock.

Dog No.	Normal values		Time in minutes	Fibrin mg.	Cells	Time in minutes	Fibrin mg.	Cells	Time in minutes	Fibrin mg.	Cells	Time in minutes	Fibrin mg.	Cells	Thrombo-plastic agent	Time of death; minutes
									Pl.							
59	354	43.0	3.0	240	46.0	7.0	182	53.7	10.0	263	54.0				Pl.	15
64	605	41.0	8.0	459	48.0	10.0	427	46.0	16.0	518	44.0				Pl.	120
69	477	49.5	4.5	209	53.0	9.0	144	49.5	13.0	168	51.0	17.0	185	50.0	1 cc. Th.	17
71	463	46.5	7.0	282	65.0	11.5	235	64.5							2 cc. Th.	12
72	540	46.0	5.0	329	56.0	10.0	303	62.5	26.0	531	50.0	70.0	516	43.5	2 cc. Th.	85
80	637	49.0	6.5	410	63.0	15.5	394	65.5	33.0	430	62.5	75.0	380	57.7	1 cc. Th.	85
82	772	51.5	10.0	359	63.0	13.0	348	63.5	22.0	345	63.2				1 cc. Th.	23
84	940	53.0	6.0	326	72.2	10.0	245	76.5							2 cc. Th.	26
85	745	55.0	14.0	630	64.7	19.0	585	66.0							2 cc. Th.	28
89	762	55.0	4.5	621	59.0	15.0	604	58.7	30.0	546	59.5	45.0	577	56.7	2 cc. Th.	65

Pl. = platelets. Th. = thromboplastin (Squibbs).

the fibrin there is a well defined rise in the hemotocrit values, frequently from 10 to 20 per cent above the normal. The maximum hematocrit readings are generally attained by the tenth minute (average on thirty dogs in fatal shock), immediately following which the fibrin generally reaches its lowest level. This would suggest that the drop in fibrin is probably due to an escape of plasma proteins incident to the increased permeability of the capillary endothelium recognized in anaphylaxis, although the possibility of its partial destruction cannot be excluded.

The following table illustrates the points in question. The fibrin is expressed in milligrams per 100 cc. of blood; the hematocrit (cells) in per cent.

2871

The blood platelets in canine anaphylaxis.

ALBERT P. KRUEGER and EDWIN W. SCHULTZ.

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.*]

It is well recognized that in canine anaphylaxis the blood generally becomes incoagulable, often remaining fluid for days. This loss of coagulability of the blood has been attributed to an excess of antithrobin or to a diminution of thromboplastin. Recently one of us noted,[1] in connection with studies on the blood fibrin in canine anaphylaxis, that the addition of a small quantity of platelets to the clotting solution, into which the oxalated anaphylactic plasma had been discharged, induced prompt coagulation in samples which otherwise (in controls) remained uncoagulated for hours, sometimes for days. This suggested the desirability of making platelet counts. While a diminution in the platelets in anaphylaxis has been reported,[2, 3] we were especially interested in the counts obtained in fatal canine anaphylaxis, particularly since changes in the coagulability of the blood are more prominent in the dog than in other animals.

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1 Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 343.

2 Archard, Ch., and Ayanud, M., *Compt. rend. Soc. de biol.*, 1909, lxvii, 83.

3 Pesci, E., *J. de physiol. et path. gen.*, 1921, xix, 242.

Of a number of current methods employed in counting platelets we found none that gave us entirely satisfactory results. We finally adopted the method of Rees and Ecker,[4] using, however, instead of their diluting fluid that recommended by Leake and Guy.[5] This combination gave us entirely satisfactory checks. The time allowed for settling in the counting chamber was reduced to a constant minimum to prevent undue evaporation of the diluting fluid and to insure strictly relative counts. The blood samples were obtained from the common carotid artery by incision. A rubber covered artery clamp was placed proximal to the opening and the blood was allowed to flow freely before the samples were taken. The animals were sensitized to horse serum,

Group I—Deep Shock Terminating Fatally.

Dog No.	Normal Platelet Count	Platelet Counts During Shock.						Time of death; minutes
1	576,000	minutes	1.5	4.5				12
		count	475,000	310,000				
2	398,000	minutes	3.0	6.0				9
		count	280,000	210,600				
3	380,000	minutes	1.0	6.5	12.0	18.0	25.0	26
		count	290,000	158,700	141,600	99,860	98,720	
4	398,800	minutes	7.0	15.0	20.0			28
		count	227,000	161,000	132,600			
5	528,000	minutes	3.5	8.5	17.5	26.5		30
		count	440,200	296,000	208,600	150,100		
6	530,100	minutes	4.5	9.0	15.0	30.0	45.0	65
		count	398,600	281,000	236,000	152,800	160,200	
7	483,000	minutes	3.0	9.0	18.0	23.0		29
		count	401,000	304,000	272,600	170,800		
8	492,000	minutes	5.0	13.0	19.0	29.0		30
		count	401,600	298,400	203,000	210,800		

Group II—Light Shock not Terminating Fatally.

1	852,800	minutes	2.0	11.0	16.0	31.0	45.0
		count	824,000	804,000	762,400	710,000	821,600
2	492,100	minutes	6.5	15.5	33.0	75.0	
		count	389,600	398,400	352,000	522,000	
3	469,000	minutes	7.0	17.0	27.0	42.0	52.0
		count	349,100	297,200	296,000	312,400	322,600

[4] Rees, H. M., and Ecker, E. E., *J. Am. Med. Assn.*, 1923, lxxx, 621.

[5] Leake, C. D., and Guy, E. F., *J. Am. Med. Assn.*, 1925, lxxxiv, 890.

0.3 cc. per kilo subcutaneously, followed on the third day with the same dose intracardially, and the shocking dose, 1 to 2 cc. per kilo, was injected intracardially.

Our results show a decrease in the platelets ranging from 47 to 71 per cent below the normal count, depending on the time at which the samples were taken. The drop is as a rule progressive, becoming more marked as the state of shock continues. In arriving at these figures the shrinkage of the blood volume and consequent relative increase of the cells during shock, was not taken into consideration. Correction for this would show a further reduction in the platelet counts. In the case of non-fatal shock the decline in platelets is not so marked. Two non-sensitized dogs injected with the equivalent amount of horse serum showed no material difference in the platelet count.

2872

Elimination of streptococci in blood stream through the biliary system in the dog.

MARY E. MATHES and EDWIN W. SCHULTZ.

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.*]

Streptococci injected into the blood stream of the dog, even in massive doses, are removed from the circulating blood with surprising rapidity. Blood cultures made at short intervals show a precipitous decline from more than ten thousand colonies per cubic centimeter to zero within four to six hours. Examination of the tissues within two or three hours show sparsely scattered cocci in the liver, spleen and lungs. In later examinations the organisms are generally difficult to find. Although the leucocyte count rises appreciably following the injections, sometimes fifty per cent or more within five hours, it is difficult to find cocci in the leucocytes in smears prepared at short intervals during the experiment.

While it cannot be denied that many of the organisms are filtered out in the capillary and sinusoidal beds and picked up by phagocytic cells, especially in the spleen, liver and bone marrow,

as well as phagocyted by the leucocytes in the circulating blood, our attention has been directed to another mechanism which may be important in the removal of the organisms. In cultures made of the urine and bile it was found that while the urine failed to reveal streptococci, the bile generally became positive. The observations were made on dogs which had been subjected to a preliminary laparotomy several days previously, at which time a small glass tube, to which rubber tubing was attached, was securely tied into the gall-bladder. The rubber tubing, about five millimeters in diameter, was brought through the laparotomy opening and the external portion carefully tied off with a number of ligatures. The wound was then closed and the animal returned to the kennels. At the time of the experiment, the bile was aspirated through the tube by means of a seriologic pipette and at about the same interval as the blood cultures were made from the heart. As a rule 0.5 cc. of bile was plated out on blood agar, each sample being taken with a fresh pipette. Hemolytic streptococci were used. The organisms generally appeared in the bile within fifteen minutes, sometimes with only a few colonies per plate, but the numbers rose steadily, occasionally attaining several hundred colonies per cubic centimeter. In one animal in which a final sample was obtained five hours after the injection there were more than 1200 organisms per cubic centimeter of bile. It has recently occurred to us that as an additional precaution we should have performed a preliminary ligation of the cystic artery, although it seems unlikely that the organisms should pass into the bile from this source, at least within the space of a few minutes. According to Herring and Simpson[1] the endothelium of the sinusoids in the liver of the dog is imperfect, being represented by partially detached cells, so that the blood flowing through the sinusoids comes in direct contact with liver cells. Furthermore, the fine branching canaliculi which permeate the liver cell communicate with the surface of the cells abutting on the sinusoids, as is evidenced by the fact that red cells may be found even in normal liver cells and that injecting fluids find their way with great ease from the sinusoids into the substance of the liver cells. It is conceivable therefore that bacteria may enter the liver cell as indicated and be eliminated therefrom by the intra- and thence the intercellular bile canaliculi.

[1] Herring, P. T., and Simpson, S., *Proc. Roy. Soc.*, Lond., 1906, B, lxxviii, 455.

2873

A striking cocaine-tyramine antagonism.

M. L. TAINTER and H. A. SHOEMAKER. (Introduced by P. J. Hanzlik).

[*From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.*]

Pharmacologically, tyramine and epinephrine appear to be similar, but not identical, in several directions. They are, of course, closely related chemically. A difference between the two drugs can be readily demonstrated in cocainized animals. Whereas ineffective doses of cocaine greatly increase (sensitize) the blood pressure response to epinephrine, they prevent (antagonize) that to tyramine in the same animal. The prevention of tyramine action by cocaine was discovered recently by us in connection with a study of tyramine action. Pending further study of its mechanism, we desire to make a preliminary report of our results at this time.

The antagonism has been demonstrated in dogs, cats and rabbits, being more marked in dogs and cats than in rabbits, whose response to tyramine is more variable. The dosage of tyramine used ranged from 0.2 to 0.5 mg. per kilo intravenously and of cocaine from 4 to 22.5 mg. per kilo subcutaneously. As a rule, the dosage of cocaine used produced little or no change in blood pressure, pulse rate, respiration and rectal temperature, and no symptoms. In about 10 minutes after the hypodermic injection of an ineffective dose of cocaine (10 mg. per kilo), the rise of blood pressure from tyramine was no longer demonstrable. The antagonism persisted from one to three hours after the administration of cocaine. This action of cocaine appeared to be rather specific, for large doses of other local anesthetics, namely procaine, butyn, and saligenin, did not prevent the tyramine rise of blood pressure in the same animals. The antagonism is not due to paralysis or depression of the sympathetics of the blood vessels, because epinephrine gave a more marked rise of blood pressure after cocaine (sensitization) than before, and stimulation of the sciatic nerve produced the usual blood pressure rise. The vessels of the perfused rabbit's ear continued to be constricted after treatment with high concentrations of cocaine. Provisionally, it appears that the antagonism is systemic, but the adrenals are not involved, since the phenomenon has been demonstrated in adrenalectomized cats. Final conclusions are reserved until the study is completed.

Southern Branch

Tulane University, October 22, 1925.

2874

A method for the determination of calcium in tissues.

R. C. CORLEY and W. DENIS.

[*From the Department of Bio-Chemistry of the School of Medicine of Tulane University, New Orleans, La.*]

The determination of calcium in tissues is almost invariably carried out on the ash. Ashing is, however, a time consuming and laborious procedure, and is in addition distinctly expensive on account of the platinum ware required.

We have, therefore, worked out a method whereby ashing is replaced by autoclave digestion. Ten grams of tissue is digested with 50 cc. of 0.1 N sodium hydroxide for two hours at 200° C. The solution is then acidified with hydrochloric acid, diluted to 60 cc., allowed to stand for at least 30 minutes, and filtered by suction through a Gooch crucible provided with an asbestos mat. An aliquot of this filtrate contained in a conical centrifuge tube is made alkaline to methyl red by the cautious addition of ammonium hydroxide and the calcium precipitated by means of ammonium oxalate. After one hour the tube is centrifuged and the supernatant liquid poured off as completely as possible, care being taken to avoid disturbing the precipitate.

The precipitate is dissolved in normal sulfuric acid and 0.1 N potassium permanganate added drop by drop until the solution is pink. After a few minutes there is added 5 cc. of water, and a sufficient amount of ammonium hydroxide to bring the solution to the neutral point of methyl red. After standing at room temperature for at least one hour the tube is centrifuged, the supernatant liquid removed, and the precipitate washed with 30 cc. of ice cold distilled water. The precipitate is then dissolved in 5 cc. of normal sulfuric acid and the calcium oxalate titrated with 0.01 N K Mn 04 in the usual manner.

2875

Calcium metabolism in tissues affected by calcium salts and ultra violet light.

W. DENIS and R. C. CORLEY.

[*From the Department of Bio-Chemistry of the School of Medicine, Tulane University, New Orleans, La.*]

Experiments have been carried out to determine whether excessive doses of calcium salts administered daily to rabbits over a considerable period could affect the calcium content of the tissues, and also as to the effect of daily exposure to ultra violet radiation with and without the ingestion of calcium salts.

Six animals were given a daily dose of 1.08 grams calcium lactate for 34 days, five received 0.5 grams calcium chloride for 40 days and six served as controls and were maintained for 35 days under the same living conditions as were the experimental animals.

In a second series eight rabbits were given calcium lactate for 31 days, eight calcium chloride for 38 days, and seven were used as controls. All of this latter group of animals received daily exposure for 30 minutes to the light of a Cooper-Hewitt mercury tungsten arc.

Calcium determinations made on the tissues and blood of these animals, indicated that there was no increase produced in the calcium content of the tissues by the administration of calcium salts either with or without the use of ultra violet light.

2876

Combined supernormal and fatigue phenomena in compressed cardiac muscle of the turtle.

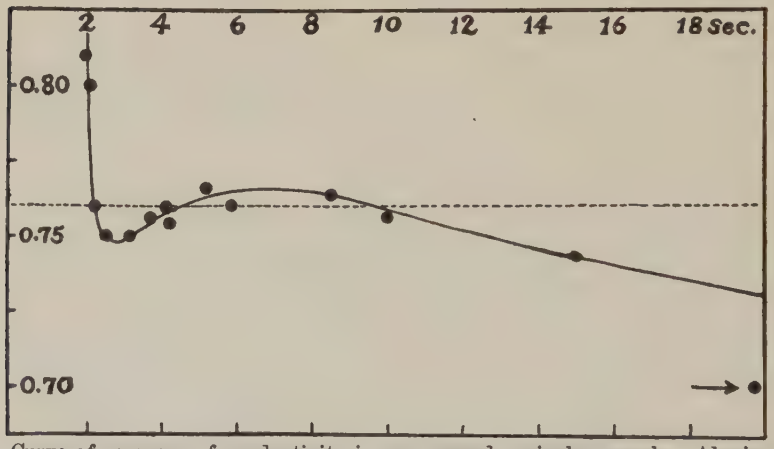
RICHARD ASHMAN and EOLA WOOLLEY.

[*From the Physiological Laboratory of the Tulane University School of Medicine, New Orleans, La.*]

One of the most characteristic features of low grade partial block, as seen clinically, is the gradual prolongation of the A-V

interval up to the time of the dropping out of a single ventricular response. This prolongation may be regarded as a fatigue effect. Furthermore, cases of partial heart block have been reported in which the recovery of conductivity passes through a supernormal phase,[1, 2] and the course of the supernormal recovery has been traced in compressed heart muscle of the turtle by one of us.[3] Therefore, since both supernormal recovery and fatigue are known to occur, separately at least, in clinical heart block cases, it appeared desirable to study the two phenomena as they occur simultaneously in the compressed turtle muscle. The results seem to us to have justified the effort, since certain relationships between the two have been discovered which could scarcely have been predicted from a study of the two phenomena separately.

In some experiments we used the atropinized, excised, bloodless hearts of *Chrysemys elegans* and *C. troosti,* rendered quiescent by removal of the sinus. In other cases, the unatropinized ventricle alone was employed, the muscle being split from the



Curve of recovery of conductivity in compressed auricular muscle. Abscissæ, intervals between auricular responses. Ordinates, the consequent A-V intervals in seconds. The point at end of arrow is at 30 sec., and is the average of 12 points, the A-V's of which varied between 0.68 and 0.71. It is supposed that if the compression had been increased to just the right degree, impulses corresponding to points above the dotted line would have been blocked, those below transmitted (see text).

[1] Lewis, T., and Master, A. M., *Heart,* 1924, xi, 371.

[2] Wilson, F. N., and Herrmann, G. R., *Arch. of Int. Med.,* 1923, xxxi, 923.

[3] Ashman, R., *Am. J. Physiol.,* 1925, lxxiv, 140.

base to near the apex. A heart clamp was placed in the A-V groove, or at the apex when the ventricle was used, and the desired degree of compression applied. The muscle was then caused to respond to induction shocks at any desired frequency. Simultaneous electrograms and myograms of the responses were recorded. Since the weather was warm, marked fatigue effects were invariably obtained. The supernormal phase was not so constant in its appearance, although shown by both types of preparation.

In the figure is shown a recovery curve of conductivity obtained when the clamp was in the A-V groove. The stimuli were applied in pairs, a 30 sec. rest being allowed after each pair. The intervals between the members of each pair were varied from 1.94 sec. up to 15.0 sec. Along the abscissæ, the time between responses is plotted. Along the ordinates, the A-V intervals in seconds. It will be seen that there is an interval, following the transmission of one impulse, during which a second impulse would have been blocked. The exact length of this interval is here undetermined. There is then rapid recovery of conductivity as determined by the duration of the A-V intervals, until the crest of the supernormal recovery curve is reached. Following this there is again depression of conductivity, succeeded in turn by the slower recovery from fatigue. Similar results were obtained with ventricular preparations (usually not atropinized since the ventricle receives no vago-inhibitory fibers).

Another experiment, typical of a number, and in which the degree of compression is greater, may be described. In this experiment (unatropinized ventricular muscle) an impulse following another at an interval shorter than 2.0 sec. was blocked; if between 2.0 and about 7.0 sec., *i. e.,* during the supernormal, it was transmitted; if between 7.0 and 28.0 sec., it was blocked; but if the impulse came later than about 28.0 sec. after the transmission of the previous one, it was transmitted. These statements require some modification for intervals up to 7.0 sec. since "treppes" and fatigue complicated the picture. Beyond 7.0 sec. there was no exception.

These experiments prove that when the supernormal recovery phase appears in a partially fatigued muscle, the conductivity after a relatively long rest may approach or exceed the conductivity at the crest of the supernormal phase, while conductivity is at other times depressed.

The relation between the "treppe" in conductivity and fatigue is illustrated in the following measurements, taken in sequence from one experiment. The numbers not in brackets represent the intervals between stimuli in seconds; those bracketed are the consequent V-V intervals, the ventricle being used. 120.0 (0.42), 7.5 (0.54), 5.5 (0.59), 15.3 (0.68), 3.0 (0.39), 2.1 (0.44) 2.2 (0.51), 2.1 (0.64), 2.4 (blocked), 2.4 (0.70), 30.0 (blocked), 4.1 (0.42), 3.9 (0.52), 4.9 (blocked), 5.9 (blocked), 4.1 (0.57). It is evident that the "treppe" is most clearly marked after a rest of moderate duration (10 to 30 sec.). After a longer rest, the V-V interval is again short and the "treppe" does not appear, although the onset of fatigue is rapid. This seems to prove a difference between the condition of the tissue immediately responsible for the fatigue, and the condition favorable for the appearance of the supernormal phase. The experiment likewise proves that the supernormal recovery of conductivity may, like the recovery of excitability, be depressed by fatigue. Certain applications of these conclusions will be found in the following communication.

2877

Periods of spontaneous rhythm in the turtle heart and their bearing upon paroxysmal tachycardia.

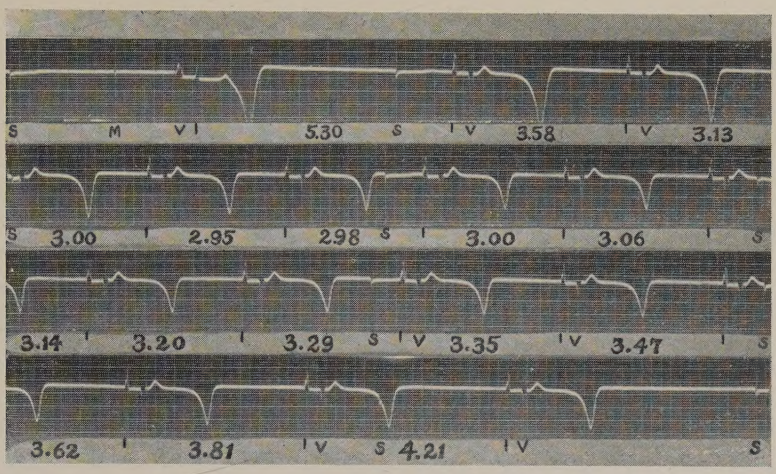
RICHARD ASHMAN and ROBERTA HAFKESBRING.

[*From the Physiological Laboratory of the Tulane University School of Medicine, New Orleans, La.*]

In frogs' hearts filled with mammalian serum and ligated between sinus venosus and auricles, or at other points, Luciani[1] observed and recorded periods of spontaneous rhythm separated by intervals of quiescence. We have obtained myograms and electrograms of the same phenomenon in turtle hearts perfused with strongly buffered, oxygenated physiological saline solutions at various H-ion concentrations. A typical period of auricular origin recorded with the string galvanometer is given in Figure 1. It will be noted that there is a rapid acceleration in the rhythm and then a more gradual retardation until the period ends.

[1] Luciani, *Human Physiology*, Eng. Trans., 1911, i, 302.

FIG. 1.



Electrogram of a complete period, initiated by a single break shock applied to the ventricle. S, the sinus negativity. The sinus was ligated off. M, the subminimal make shock. V, beginning of ventricular response. The short vertical lines below the electrogram indicate the beginning of auricular negativity The numbers, the interauricular intervals.

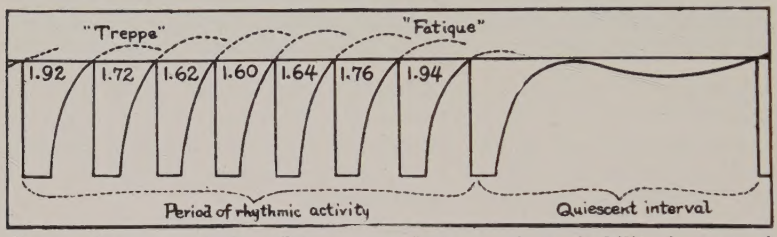
Certain peculiarities in the time relations of the cycles during a period, and of the intervals between spontaneously initiated periods, suggested that their origin and course might readily be explained on the basis of a few well supported assumptions. *First,* that the impulses are discharged from an ectopic focus. Since the sinus is ligated off and since the time relations and electrograms seem to prove that the period is not due to circus contractions, there must be an ectopic focus. *Second,* that the impulse discharge depends upon the attainment of a sufficient degree of excitability in the focus. This is probable, for although highly excitable tissues are not necessarily spontaneously active, no one is likely to deny that the excitability is one determining factor where conditions are otherwise favorable to impulse discharge.[2] *Third,* that the returning excitability after each discharge passes through a supernormal phase. Adrian[3] has shown that nerve and muscle bathed in faintly acid solutions do show a supernormal recovery, *i. e.,* the excitability for a certain time

[2] Andrus, E. C., and Carter, E. P., *Heart,* 1924, xi, 97.

[3] Adrian, E. D., *J. Physiol.,* 1920, liv, 91.

after the response exceeds the resting (and depressed) excitability. *Fourth,* that there is a "treppe" in excitability for the first several recoveries of the period. It has been demonstrated that a "treppe" in *conductivity* may appear under suitable conditions in the compressed cardiac muscle of the turtle.[4] If, as is probable, conductivity is an aspect of excitability, we have here evidence for a "treppe" in excitability. *Fifth,* that the continued activity of the ectopic focus leads to its own fatigue. That the supernormal conductivity is depressed by fatigue is shown in a previous report in this number of the PROCEEDINGS. *Sixth,* that recovery from fatigue occurs during the quiescent interval between periods.

FIG. 2.



Schematic representation of the assumed changes in excitability in an auricular ectopic focus during and following a period of activity initiated in this case by a single induction shock applied to the ventricle. The numbers give the intervals between auricular beats. The heavy vertical lines represent the instants of impulse discharge. The short horizontal lines, the absolute refractory periods of the focus. The curves, the course of recovery of excitability in the focus. The continuous horizontal line, the threshold for impulse discharge. The dotted portions of the curves, the assumed course of recovery had the threshold been high enough to prevent impulse discharge. For convenience of representation, the quiescent interval is considerably shortened.

Figure 2 shows the postulated course of excitability during a period of activity and during a quiescent interval. With the above six points in mind, a study of this figure will make clear the mechanism which we suppose underlies the periodic rhythm in the turtle hearts in our experiments. Unless a supernormal phase and fatigue are factors involved it appears impossible to account for the sudden onset and end of the periods. In the absence of a supernormal the heart would presumably beat slowly and regularly.

[4] Ashman, R., *Am. J. Physiol.,* 1925, lxxiv, 140.

We have as yet found no definite relation between the periodicity and the pH of the perfusate. In view of Adrian's work, this is admittedly a difficulty. But there is no guarantee that the pH of the perfusate impresses itself upon all parts of the myocardium. Further, in each experiment, one auricle was not freely perfused and usually the periods were of auricular origin. However, in one or two instances the periods were of ventricular or junctional origin while the pH was 7.6.

Since the periods appeared in all our hearts which were perfused and rendered quiescent by ligation, it is to be anticipated that more than one ectopic focus might arise in the same heart. We have one excellent example of this sort, one focus being auricular, the other ventricular. Here the slower auricular focus was quickest to recover from fatigue and started the period. After it had discharged two or three impulses the more rapid ventricular focus, reaching its threshold by means of a "treppe", took over the rôle of pacemaker, but was soon fatigued. Then the auricular focus, not yet fatigued, was again able to initiate impulses and continued the period, with slower rhythm, to its conclusion.

Most interesting, because of its possible bearing on the problems of paroxysmal tachycardia and of ectopic beats in the human heart, was the effect of slow, rhythmic, electrical stimulation during the intervals between the periods of spontaneous rhythm. In general, if the rate of such stimulation was very slow the next spontaneous paroxysm was caused to appear earlier, but it was of shorter duration and of slower rhythm than the paroxysm appearing after complete rest. When the stimulation was somewhat faster, the time of appearance of the period was at the expected moment or somewhat delayed, the initial acceleration was less marked, and the duration was still further curtailed. Still more frequent electrical stimulation caused the period to appear still later and it was often limited to a *single ectopic beat.* Stimulation rapid enough to prevent recovery from fatigue completely suppressed the spontaneous activity.

It is thus clear that if an ectopic focus in the human heart possessed properties similar to those of the focus in the turtle hearts, it might, as permitted by conditions of nerve influence, sinus rhythm, and degree of supernormal recovery, discharge either single ectopic impulses, multiple ectopic impulses, or series of impulses producing paroxysms of tachycardia. It is to be

stressed that the ectopic focus is not in such cases to be regarded as a blocked-off focus, continuously rhythmically active at its own inherent rate,[5] but as a potential pacemaker, normally responding to every sinus impulse until conditions favor its assumption of the rôle of actual pacemaker. And it is further to be emphasized that a *very gradual* change in the condition of the focus could lead to the sudden onset or end of a paroxysm. We do not suppose, however, that all ectopic beats or all paroxysms of tachycardia are to be thus explained.

[5] Kaufmann, R., and Rothberger, C. J., *Zeitsch, f. d. ges. exper. Med.*, 1920, xi, 40.